IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of: R. Rogers Yocum *et al.*

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For: *Methods and Microorganisms for Production of Panto-Compounds*

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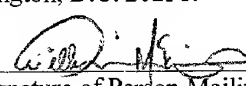
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PRELIMINARY AMENDMENT

Dear Sir:

Prior to examination of the above-identified application, please amend the application as follows:

<u>In the Claims:</u>

Please cancel claim 3-6, 8-11, 13, 45, 54-61, 63-66, 76-81, 84-87, 89, 91-96, 100-101, 103, 105 and 107, without prejudice.

Please amend claims 12, 20-25, 27-28, 33 and 46-50, as follows:

12. (Amended) The method of [any one of claims 7 to 11] claim 7, wherein the KPAR-O microorganism further overexpresses at least one pantothenate biosynthetic enzyme in addition to overexpressing ketopantoate reductase.

20. (Amended) The method of [any one of claims 14 to 19] claim 14 or 19, wherein the microorganism overexpresses acetohydroxyacid synthetase or is transformed with a vector comprising an *ilvBN* nucleic acid sequence or an *alsS* sequence.

21. (Amended) The method of [any one of claims 14 to 19] claim 14 or 19, wherein the microorganism overexpresses acetohydroxyacid isomeroreductase or is transformed with a vector comprising an *ilvC* nucleic acid sequence.

22. (Amended) The method of [any one of claims 14 to 19] claim 14 or 19, wherein the microorganism overexpresses dihydroxyacid dehydratase or is transformed with a vector comprising an *ilvD* nucleic acid sequence.

23. (Amended) The method of [any one of claims 19 to 22] claim 19, wherein the microorganism overexpresses aspartate-α-decarboxylase or is transformed with a vector comprising a *panD* nucleic acid sequence.

24. (Amended) The method of [any one of claims 14 to 19] claim 14 or 19, wherein the microorganism further has a deregulated pantothenate biosynthetic pathway.

25. (Amended) The method of [any one of claims 14 to 19] claim 14 or 19, wherein the microorganism further has at least one mutant gene selected from the group consisting of a mutant *avtA* gene, a mutant *ilvE* gene, a mutant *ansB* gene and a mutant *alsD* gene.

27. (Amended) The method of claim 24 [or 26], wherein the microorganism is transformed with a vector comprising a *panBCD* nucleic acid sequence or a vector comprising a *panE1* nucleic acid sequence.

28. (Amended) The method of [any one of claims 14 to 19] claim 14 or 19, wherein pantothenate is produced at a level selected from the group consisting of a level greater than 10g/L, a level greater than 20g/L and a level greater than 40g/L.

33. (Amended) The method of claim 24 [or 26], wherein the microorganism overexpresses any of ketopantoate hydroxymethyltransferase, ketopantoate reductase, pantothenate synthetase and aspartate-α-decarboxylase derived from *Bacillus*.

46. (Amended) The method of [any one of claims 39 to 44] claim 39 or 41, wherein said panto-compound is pantothenate.

47. (Amended) The method of [any one of claims 39 to 44] claim 39 or 41, wherein said panto-compound is produced at a level selected from the group consisting of a level greater than 10g/L, a level greater than 20g/L and a level greater than 40g/L.

48. (Amended) The method of [any one of claims 39 to 44] claim 39 or 41, wherein said recombinant microorganism further has a deregulated pantothenate biosynthetic pathway or further has a deregulated isoleucine-valine (*ilv*) biosynthetic pathway.

49. (Amended) The method of [any one of claims 39 to 44] claim 39 or 41, wherein said recombinant microorganism further overexpresses *panD* and *panE*.

50. (Amended) The method of [any one of claims 39 to 44] claim 39 or 41, wherein said recombinant microorganism further has at least one mutant gene selected from the group consisting of a mutant *avtA* gene, a mutant *ilvE* gene, a mutant *ansB* gene and a mutant *alsD* gene.
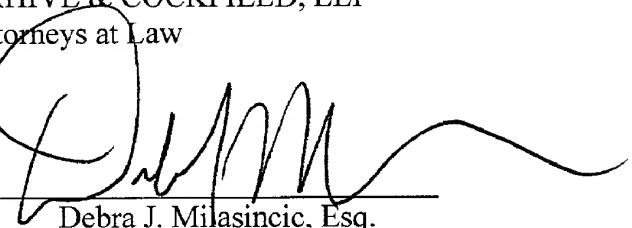
REMARKS

Applicants have attached herewith APPENDIX A setting forth the claims that will be pending after entry of the instant amendment. No new matter has been added to the application.

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APPENDIX A

1.   A method of producing a panto-compound comprising culturing a microorganism which overexpresses at least one *Bacillus* pantothenate biosynthetic enzyme under conditions such that the panto-compound is produced.

2.   The method of claim 1, wherein the microorganism overexpresses at least one *Bacillus subtilis* pantothenate biosynthetic enzyme.

7.   A method of producing a panto-compound comprising culturing a ketopantoate reductase-overexpressing (KPAR-O) microorganism under conditions such that the panto-compound is produced.

12.   The method of claim 7, wherein the KPAR-O microorganism further overexpresses at least one pantothenate biosynthetic enzyme in addition to overexpressing ketopantoate reductase.

14.   A method of producing pantothenate in a manner independent of precursor feed comprising culturing an aspartate-α-decarboxylase-overexpressing (AαD-O) microorganism having a deregulated isoleucine-valine (*ilv*) pathway under conditions such that pantothenate is produced.

15.   A method of producing at least 2 g/L pantothenate in a manner independent of aspartate or β-alanine feed comprising culturing an aspartate-α-decarboxylase-overexpressing (AαD-O) microorganism under conditions such that pantothenate is produced.

16.   A method of producing at least 2 g/L pantothenate in a manner independent of valine or α-ketoisovalerate feed comprising culturing a microorganism having a deregulated isoleucine-valine (*ilv*) biosynthetic pathway under conditions such that pantothenate is produced.

17.   A method of producing at least 30 g/L pantothenate in a manner independent of aspartate or β-alanine feed comprising culturing an aspartate-α-

decarboxylase-overexpressing (AαD-O) microorganism under conditions such that pantothenate is produced.

18.   A method of producing at least 30 g/L pantothenate in a manner independent of valine or α-ketoisovalerate feed comprising culturing a microorganism having a deregulated isoleucine-valine (*ilv*) biosynthetic pathway under conditions such that pantothenate is produced.

19.   A β-alanine independent high yield production method for producing pantothenate comprising culturing a manipulated microorganism under conditions such that pantothenate is produced at a significantly high yield.

20.   The method of claim 14 or 19, wherein the microorganism overexpresses acetohydroxyacid synthetase or is transformed with a vector comprising an *ilvBN* nucleic acid sequence or an *alsS* sequence.

21.   The method of claim 14 or 19, wherein the microorganism overexpresses acetohydroxyacid isomeroreductase or is transformed with a vector comprising an *ilvC* nucleic acid sequence.

22.   The method of claim 14 or 19, wherein the microorganism overexpresses dihydroxyacid dehydratase or is transformed with a vector comprising an *ilvD* nucleic acid sequence.

23.   The method of claim 19, wherein the microorganism overexpresses aspartate-α-decarboxylase or is transformed with a vector comprising a *panD* nucleic acid sequence.

24.   The method of claim 14 or 19, wherein the microorganism further has a deregulated pantothenate biosynthetic pathway.

25.   The method of claim 14 or 19, wherein the microorganism further has at least one mutant gene selected from the group consisting of a mutant *avtA* gene, a mutant *ilvE* gene, a mutant *ansB* gene and a mutant *alsD* gene.

26.   The method of claim 24, wherein the microorganism overexpresses any of ketopantoate hydroxymethyltransferase, ketopantoate reductase, pantothenate synthetase and aspartate-α-decarboxylase.

27.   The method of claim 24 , wherein the microorganism is transformed with a vector comprising a *panBCD* nucleic acid sequence or a vector comprising a *panE1* nucleic acid sequence.

28.   The method of claim 14 or 19, wherein pantothenate is produced at a level selected from the group consisting of a level greater than 10g/L, a level greater than 20g/L and a level greater than 40g/L.

29.   The method of claim 20, wherein the microorganism overexpresses acetohydroxyacid synthetase derived from *Bacillus* or is transformed with a vector comprising an *ilvBN* nucleic acid sequence or an *alsS* nucleic acid sequence derived from *Bacillus*.

30.   The method of claim 21, wherein the microorganism overexpresses acetohydroxyacid isomeroreductase derived from *Bacillus* or is transformed with a vector comprising an *ilvC* nucleic acid sequence derived from *Bacillus*.

31.   The method of claim 22, wherein the microorganism overexpresses dihydroxyacid dehydratase derived from *Bacillus* or is transformed with a vector comprising av *ilvD* nucleic acid sequence derived from *Bacillus*.

32.   The method of claim 23, wherein the microorganism overexpresses aspartate-α-decarboxylase derived from *Bacillus* or is transformed with a vector comprising a *panD* nucleic acid sequence derived from *Bacillus*.

33.   The method of claim 24 [or 26], wherein the microorganism overexpresses any of ketopantoate hydroxymethyltransferase, ketopantoate reductase, pantothenate synthetase and aspartate-α-decarboxylase derived from *Bacillus*.

34.   The method of claim 27, wherein the vector comprises a *panBCD* nucleic acid sequence or a *panE1* nucleic acid sequence derived from *Bacillus.*

35.   A method of producing a panto-compound comprising contacting a composition comprising at least one pantothenate biosynthesis pathway precursor or isoleucine-valine biosynthesis pathway precursor with at least one isolated *Bacillus* enzyme selected from the group consisting of ketopantoate hydroxymethyltransferase, ketopantoate reductase, pantothenate synthetase and aspartate-α-decarboxylase, under conditions such that the panto-compound is produced.

36.   A method of producing β-alanine comprising culturing an aspartate-α-decarboxylase-overexpressing (AαD-O) microorganism under conditions such that β-alanine is produced.

37.   The method of claim 36, wherein the AαD-O microorganism has a mutation in a nucleic acid sequence encoding a pantothenate biosynthetic enzyme selected from the group consisting of ketopantoate hydroxymethyltransferase, ketopantoate reductase and pantothenate synthetase.

38.   A method of producing β-alanine comprising contacting a composition comprising aspartate with an isolated *Bacillus* aspartate-α-decarboxylase enzyme under conditions such that β-alanine is produced.

39.   A method for enhancing production of a panto-compound comprising culturing a mutant microorganism having a mutant *coaX* gene under conditions such that the panto-compound production is enhanced.

40.   The method of claim 39, wherein said recombinant microorganism has a mutant *coaA* gene.

41.   A method of producing a panto-compound comprising a pantothenate kinase mutant microorganism under conditions such that the panto-compound is produced at a significantly high yield.

42. The method of claim 41, wherein said mutant microorganism has a mutant *coaA* gene.

43. The method of claim 41, wherein said mutant microorganism has a mutant *coaX* gene.

44. The method of claim 41, where said mutant microorganism has a mutant *coaA* and *coaX* gene.

46. The method of claim 39 or 41, wherein said panto-compound is pantothenate.

47. The method of claim 39 or 41, wherein said panto-compound is produced at a level selected from the group consisting of a level greater than 10g/L, a level greater than 20g/L and a level greater than 40g/L.

48. The method of claim 39 or 41, wherein said recombinant microorganism further has a deregulated pantothenate biosynthetic pathway or further has a deregulated isoleucine-valine (*ilv*) biosynthetic pathway.

49. The method of claim 39 or 41, wherein said recombinant microorganism further overexpresses *panD* and *panE*.

50. The method of claim 39 or 41, wherein said recombinant microorganism further has at least one mutant gene selected from the group consisting of a mutant *avtA* gene, a mutant *ilvE* gene, a mutant *ansB* gene and a mutant *alsD* gene.

51. A method for enhancing production of a panto-compound comprising culturing a microorganism that has a deregulated pantothenate biosynthetic pathway and that also has a mutation that results in reduced pantothenate kinase activity under conditions such that the panto-compound production is enhanced.

52. A method for identifying compounds which modulate pantothenate kinase activity comprising contacting a recombinant cell expressing pantothenate kinase

encoded by the *coaX* gene with a test compound and determining the ability of the test compound to modulate pantothenate kinase activity in said cell.

53.   The method of claim 52, wherein said cell further comprises a mutant *coaA* gene encoding a pantothenate kinase having reduced activity.

62.   A recombinant microorganism which overexpresses at least one *Bacillus* pantothenate biosynthetic enzyme.

67.   A recombinant microorganism having a mutant *coaX* gene, said mutant *coaX* gene encoding reduced pantothenate kinase activity in said microorganism.

68.   The recombinant microorganism of claim 67 further having a mutant *coaA* gene, said mutant *coaA* gene encoding reduced pantothenate kinase activity in said microorganism.

69.   A recombinant microorganism having a mutant *coaX* gene and optionally having a mutant *coaA* gene, said mutant microorganism having reduced pantothenate kinase activity as compared to a microorganism having wild-type *coaA* and *coaX* genes.

70.   A recombinant microorganism comprising a vector comprising an isolated *coaX* gene.

71.   A recombinant microorganism that overproduces a panto-compound, the microorganism having a deregulated pantothenate biosynthetic pathway and having at least one mutation that results in a decrease in the capacity of the microorganism to synthesize Coenzyme A (CoA).

72.   The recombinant microorganism of claim 71, having at least one mutation that results in a reduced level of pantothenate kinase activity.

73.   The recombinant microorganism of claim 72, having a mutation in a *coaA* gene, or homologue thereof, that results in a reduced level of CoaA enzyme activity.

74.   The recombinant microorganism of claim 72, having a mutation in a *coaX* gene, or homologue thereof, that results in a reduced level of CoaX enzyme activity.

75.   The recombinant microorganism of claim 72, having a mutation in a *coaA* gene, or homologue thereof, and having a mutation in a *coaX* gene, or homologue thereof, the mutations resulting in reduced levels of CoaA enzyme activity and reduced CoaX enzyme activity.

82.   A recombinant microorganism selected from the group consisting of PA221, PA235, PA236, PA313, PA410, PA402, PA403, PA411, PA412, PA413, PA303, PA327, PA328, PA401, PA340, PA342, PA404, PA405, PA374, PA354, PA365, PA377, PA651 and PA824.

83.   A recombinant vector for use in the production of panto-compounds comprising a nucleic acid sequence which encodes at least one *Bacillus* pantothenate biosynthetic enzyme operably linked to regulatory sequences.

88.   A vector comprising a mutant *coaX* gene, said mutant encoding a pantothenate kinase enzyme having reduced activity.

90.   A vector comprising an isolated *Bacillus coaX* gene.

97.   A vector selected from the group consisting of pAN004, pAN005, pAN006, pAN236, pAN423, pAN428, pAN429, pAN441, pAN442, pAN443, pAN251, pAN267, pAN256, pAN257, pAN263, pAN240, pAN294, pAN296, pAN336, pAN341 and pAN342.

99.   An isolated nucleic acid molecule which encodes at least one *Bacillus* pantothenate biosynthetic gene.

102.   An isolated *Bacillus* pantothenate biosynthetic enzyme polypeptide.

104.   An isolated *Bacillus* ketopantoate reductase polypeptide.

106.   An isolated *Bacillus* aspartate-α-decarboxylase polypeptide.

108.   An isolated nucleic acid molecule comprising a mutant *coa*X gene.

109.   An isolated nucleic acid molecule comprising a *coaX* gene.

110.   An isolated pantothenate kinase protein encoded by a *coaX* gene.

METHODS AND MICROORGANISMS FOR
PRODUCTION OF PANTO-COMPOUNDS

Related Applications

5   The instant application is a continuation-in-part of U.S. Patent
Application Serial No. 09/400,494, filed September 21, 1999 (pending). The instant
application also claims the benefit of prior filed provisional U.S. Patent Application
Serial No. 60/210,072, filed June 7, 2000, prior filed provisional U.S. Patent Application
Serial No. 60/221,938, filed July 28, 2000 and prior filed provisional U.S. Patent
10   Application Serial No. 60/227,860, filed August 24, 2000. The entire content of the
above-referenced patent applications is incorporated herein by this reference.

Background of the Invention

                Pantothenate, also known as pantothenic acid or vitamin B5, is a member
15   of the B complex of vitamins and is a nutritional requirement for mammals, including
livestock and humans (e.g., from food sources, as a water soluble vitamin supplement or
as a feed additive). In cells, pantothenate is used primarily for the biosynthesis of
coenzyme A (CoA) and acyl carrier protein (ACP). These coenzymes function in the
metabolism of acyl moieties which form thioesters with the sulfhydryl group of the 4'-
20   phosphopantetheine portion of these molecules. These coenzymes are essential in all
cells, participating in over 100 different intermediary reactions in cellular metabolism.
                The conventional means of synthesizing pantothenate (in particular, the
bioactive D isomer) is via chemical synthesis from bulk chemicals, a process which is
hampered by excessive substrate cost as well as the requirement for optical resolution of
25   racemic intermediates (e.g., resolution of DL-pantolactone to obtain D-pantolactone for
chemical condensation with β-alanine). Accordingly, researchers have recently looked
to bacterial or microbial systems that produce enzymes useful in pantothenate
biosynthesis processes (as bacteria are themselves capable of synthesizing
pantothenate). In particular, bioconversion processes have been evaluated as a means of
30   favoring production of the D isomer of pantothenic acid, e.g., using microorganisms
which selectively hydrolyze a DL-pantothenic acid ester to D-pantothenic acid;
microorganisms which selectively decompose L-pantolactone resulting in D-
pantolactone alone; and microorganisms which selectively hydrolyze DL-pantolactone
to D-pantoic acid.
35   There is still, however, significant need for improved pantothenate
production processes, in particular, for processes requiring reduced quantities of
substrates and/or less expensive substrates. To this end, methods of direct microbial

synthesis have recently been examined as a means of improving D-pantothenate production. In microbes, pantothenate biosynthetis is a multistep pathway resulting in condensation of pantoate (derived from α-ketoisovalerate) and β-alanine to form D-pantothenate. The isoleucine-valine (*ilv*) pathway biosynthetic enzymes,

5   acetohydroxyacid synthetase (the *ilvBN* or *alsS* gene product), acetohydroxyacid isomeroreductase (the *ilvC* gene product) and dihydroxyacid dehydratase (the *ilvD* gene product) catalyze the conversion of pyruvate to α-ketoisovalerate. The reactions are further catalyzed by the pantothenate (*pan*) pathway biosynthetic enzymes ketopantoate hydroxymethyltransferase (the *panB* gene product), ketopantoate reductase (the *panE*

10   gene product), aspartate-α-decarboxylase (the *panD* gene product) and pantothenate synthetase (the *panC* gene product).

   The genes encoding the enzymes involved in the biosynthesis of pantothenic acid in *Salmonella typhimurium* and *Escherichia coli* have recently been identified and characterized (Frodyma and Downs (1998) *J. Biol. Chem.* 273:5572-5576

15   and Jackowski (1996) pp. 687-694, *In* Neidhardt *et al* (ed.) *Escherichia coli* and *Salmonella*: Cellular and Molecular Biology, 2nd ed. *Am. Soc. Microbiol.* Wash, D.C). In *E. coli*, for example, the biosynthesis of pantothenic acid consists of four key steps. The first reaction is catalyzed by the *panB* gene product, ketopantoate hydroxymethyltransferase, and uses the L-valine intermediate α-ketoisovalerate to

20   generate ketopantoate, which is subsequently reduced to pantoate by the *panE* gene product, ketopantoate reductase. The *panD* gene product, aspartate-α-decarboxylase, generates β-alanine from aspartate. The *panC* gene product, pantothenate synthetase, subsequently ligates β-alanine with pantoate to yield D-pantothenate.

   The authors Dusch *et al.* described the identification of the

25   *Corynebacterium glutamicum panD* gene and reported that expression of the *C. glutamicum panD* gene in *E. coli* yielded a strain producing pantothenate with a specific productivity of 140 ng of pantothenate per mg (dry weight) per hour. (Dusch *et al.* (1999) *Appl. Environ. Microbiol.* 65:1530-1539).

   The authors Sahm and Eggeling have further identified the

30   *Corynebacterium glutamicum panB* and *pan C* genes and have described a genetically engineered strain of *C. glutamicum* which overexpresses the *panBC* genes (Sahm and Eggeling (1999) *Appl. Environ. Microbiol.* 65:1973-1979). The engineered strain produces pantothenate, however, it was necessary to overexpress the genes responsible for α-ketoisovalerate production in the host organism in order that pantothenic acid

35   production could be detected. Moreover, without the addition of β-alanine, no substantial amounts of pantothenate accumulated with the strain constructed.

Likewise, a method of producing D-pantothenic acid has been described that takes advantage of a sodium salicylate resistant mutant strain of *E. coli* which produces D-pantothenic acid when cultured in the presence of β-alanine (U.S. Patent No. 5,518,906). Generation of *E. coli* strains resistant to α-ketoisovaleric acid and/or α-ketobutyric acid, and/or α-aminobutyric acid, and/or β-hydroxyaspartic acid and/or O-methyl-threonine, in addition to salicylic acid, further increased pantothenic acid production. Moreover, transformation of a plasmid DNA carrying the *panB, panC* and *panD* genes into the salicylic acid resistant mutant strain resulted in increased pantothenate production, however, up to 20 g/L β-alanine or more was fed in the examples given. The *panB-panC-panD* genes are clustered on the *E. coli* chromosome.

Finally, a method of producing D-pantothenic acid has been described which utilizes a salicylic acid-resistant, α-ketoisovalerate-resistant, α-ketobutyrate-resistant, β-hydroxyaspartate-resistant, o-methylthreonine-resistent *E. coli* strain transformed with pantothenate biosynthesis gene-containing DNA fragments and/or branched amino acid biosynthesis gene-containing DNA fragments and cultured in the presence of β-alanine (U.S. Patent No. 5,932,457).

Pantothenate production in bacteria results from the condensation of pantoate and β-alanine and involves the pantothenate biosynthetic enzymes ketopantoate hydroxymethyltransferase (the *panB* gene product), ketopantoate reductase (the *panE* gene product), aspartate-α-decarboxylase (the *panD* gene product) and pantothenate synthetase (the *panC* gene product). Although pantothenate is biologically active as a vitamin, it is further metabolized in all cells to Coenzyme A (CoA) which participates as an acyl group carrier in the tricarboxylic acid (TCA) cycle, fatty acid metabolism and numerous other reactions of intermediary metabolism. The initial (and possibly rate-controlling) step in the conversion of pantothenate to Coenzyme A (CoA) is phosphorylation of pantothenate by pantothenate kinase. A pantothenate kinase activity was first identified in *Salmonella typhimurium* by screening for temperature-sensitive mutants which synthesized CoA at permissive temperatures but excreted pantothenate at non-permissive temperatures. The mutations were mapped in the *Salmonella* chromosome and the genetic locus was designated *coaA*. The gene encodes the enzyme that catalyzes the first step in the biosynthesis of coenzyme A from pantothenate (Dunn and Snell (1979) *J. Bacteriol.* 140:805-808). *Escherichia coli* temperature sensitive mutants have also been isolated and characterized (Vallari and Rock (1987) *J. Bacteriol.* 169:5795-5800). These mutants (named *coaA15(Ts)*) are defective in the conversion of pantothenate to CoA and further exhibit a temperature-sensitive growth phenotype, indicating that pantothenate kinase activity is essential for growth. Moreover, it was

noted that CoA inhibited pantothenate kinase activity to the same degree in the mutant as compared to the wild-type enzyme.

Feedback resistant *E. coli* mutants (named *coaA16(Fr)*) have also been isolated that posses a pantothenate kinase activity that is refractory to feedback

5   inhibition by CoA (Vallari and Jackowski (1988) *J. Bacteriol.* 170:3961-3966). The mutation responsible for the reversion is, suprisingly, not genetically linked to the *coaA* gene by transduction. Additional data described therein support the view that the total cellular CoA content is controlled by both modulation of biosynthesis at the pantothenate kinase step and possibly by degradation of CoA to 4'-phosphopantetheine.

10   The wild-type *E. coli coaA* gene was cloned by functional complementation of *E. coli* temperature-sensitive mutants. The sequence of the wild-type gene was determined (Song and Jackowski (1992) *J. Bacteriol.* 174:6411-6417 and Flamm *et al.* (1988) *Gene (Amst.)* 74:555-558). Strains containing multiple copies of the *coaA* gene possessed 76-fold higher specific activity of pantothenate kinase,

15   however, there was only a 2.7-fold increase in the steady state level of CoA (Song and Jackowski, *supra*). It has further been reported that the prokaryotic enzyme (encoded by *coaA* in *E.coli* and a variety of other microorganisms) is feedback inhibited by CoA both *in vivo* and *in vitro* with CoA being about five times more potent than acetyl-CoA in inhibiting the enzyme (*Song and Jackowski, supra* and Vallari *et al., supra*). Moreover,

20   it has been reported that the *panB* gene product in *E. coli* is inhibited by CoA (Powers and Snell (1976) *J. Biol. Chem.* 251:3786-3793). These data further support the view that feedback inhibition of pantothenate kinase activity is a critical factor controlling intracellular CoA concentration.

Using standard search and alignment tools, *coaA* homologues have been

25   identified in *Hemophilus influenzae, Mycobacterium tuberculosis, Vibrio cholerae, Streptococcus pyogenes* and *Bacillus subtilis*. By contrast, proteins with significant similarity could not be identified in eukaryotic cells including *Saccharomyces cerevisiae* or in mammalian expressed sequence tag (EST) databases. Using a genetic selection strategy, a cDNA encoding pantothenate kinase activity has recently been identified

30   from *Aspergillus nidulans* (Calder *et al.* (1999) *J. Biol. Chem.* 274:2014-2020). The eukaryotic pantothenate kinase gene (*panK*) has distinct primary structure and unique regulatory properties that clearly distinguish it from its prokaryotic counterpart. A mammalian pantothenate kinase gene (*mpanK1α*) has also been isolated which encodes a protein having homology to the *A. nidulans* PanK protein and to the predicted gene

35   product of GenBank™ Accession Number 927798 identified in the *S. cerevisiae* genome (Rock *et al.* (2000) *J. Biol. Chem.* 275:1377-1383).

Summary of the Invention

The present invention is based, at least in part, on the discovery of key enzyme-encoding genes of the pantothenate biosynthetic pathway in *Bacillus subtilis*.

5   In particular, the present inventors have identified the *panE* gene of *B. subtilis*. Overexpression or deregulation of the *panE* gene in *B. subtilis* results in enhanced production of the *panE* gene product, ketopantoate reductase, further resulting in increased production of pantothenate. Likewise, mutations in this gene reduce pantothenate production in *B. subtilis* >90%. The present inventors have further

10   identified the presumptive *panBCD* operon in *B. subtilis*, overexpression or deregulation of which results in increased pantothenate production. The present inventors have further demonstrated that overexpression or deregulation of the *panD* gene in *B. subtilis* (resulting in enhanced production of the *panD* gene product, aspartate-α-decarboxylase) further results in increased production of pantothenate, in

15   particular, in combination with deregulation of genes encoding key enzymes of the isoleucine-valine (*ilv*) biosynthetic pathway.

Accordingly, the present invention features methods of producing pantothenate, as well as other compounds of the pantothenate biosynthetic pathway (*e.g.*, ketopantoate, pantoate and β-alanine), termed "panto-compounds" herein, using

20   microorganisms in which the pantothenate biosynthetic pathway and/or isoleucine-valine biosynthetic pathway has been manipulated such that pantothenate or other desired panto-compounds are produced. In one embodiment, the invention features a method of producing a panto-compound (*e.g.*, pantothenate or pantoate) that involves culturing a microorganism which overexpresses the *panE* gene product, ketopantoate

25   reductase, also referred to herein as a ketopantoate reductase-overexpressing or "KPAR-O" microorganism, under conditions such that the panto-compound (*e.g.*, pantothenate or pantoate) is produced. In another embodiment, the present invention features a method of producing panto-compounds (*e.g.*, pantothenate or pantoate) which includes culturing a microorganism which overexpresses at least one

30   pantothenate biosynthetic enzyme (*e.g.*, at least one of the *panB*, *panC* or *panD* gene products), preferably in a KPAR-O microorganism, under conditions such that the panto-compound (*e.g.*, pantothenate or pantoate) is produced.

Yet another aspect of the invention features methods of producing panto-compounds which are independent of the need to feed precursors (*e.g.*, β-alanine or

35   aspartate and/or α-ketoisovalerate or valine). In one embodiment, the invention features a method of producing pantothenate in a manner independent of precursor feed that includes culturing an aspartate-α-decarboxylase-overexpressing (AαD-O)

microorganism having a deregulated isoleucine-valine (*ilv*) pathway under conditions such that pantothenate is produced. In another embodiment, the invention features a method of producing pantothenate in a manner independent of precursor feed that includes culturing an AαD-O microorganism having a deregulated pantothenate (*pan*)

5   pathway and a deregulated isoleucine-valine (*ilv*) pathway, under conditions such that pantothenate is produced. In another embodiment, the invention features a method of producing pantothenate in a manner independent of aspartate or β-alanine feed that includes culturing an AαD-O microorganism under conditions such that pantothenate is produced. In another embodiment, the invention features a method of producing

10   pantothenate in a manner independent of valine or α-ketoisovalerate feed that includes culturing a microorganism having a deregulated isoleucine-valine (*ilv*) biosynthetic pathway under conditions such that pantothenate is produced. In yet another embodiment, the invention features a high yield production method for producing pantothenate that includes culturing a manipulated microorganism under conditions

15   such that pantothenate is produced at a significantly high yield (*e.g.*, at a level greater than 10 g/L, 20 g/L, 30 g/L or 40g/L).

        The methods of the present invention further feature microorganisms that overexpresses acetohydroxyacid synthetase or acetohydroxyacid isomeroreductase (*e.g.*, microorganisms transformed with a vector that includes an *ilvBNC* nucleic acid

20   sequence), microorganisms that overexpresses dihydroxyacid dehydratase (*e.g.*, microorganisms transformed with a vector that includes an *ilvD* nucleic acid sequence), microorganisms that overexpresses aspartate-α-decarboxylase (*e.g.*, microorganisms transformed with a vector that includes a *panD* nucleic acid sequence), microorganisms having a deregulated isoleucine-valine (*ilv*) biosynthetic pathway and microorganisms

25   having a deregulated pantothenate biosynthetic pathway (*e.g.*, microorganisms that overexpress any of ketopantoate hydroxymethyltransferase, ketopantoate reductase, pantothenate synthetase and aspartate-α-decarboxylase, for example, microorganisms transformed with a vector comprising a *panBCD* nucleic acid sequence or a vector comprising a *panE1* nucleic acid sequence). In one embodiment, the recombinant

30   microorganism is Gram positive (*e.g.*, microorganisms belonging to the genus *Bacillus*, *Cornyebacterium*, *Lactobacillus*, *Lactococci* or *Streptomyces*). In another embodiment, the recombinant microorganism is Gram negative. Particularly preferred is a *Bacillus* recombinant microorganism (*e.g.*, *Bacillus licheniformis*, *Bacillus amyloliquefaciens*, *Bacillus subtilis*, *Bacillus pumilus*, *Bacillus halodurans*, and the like). Recombinant

35   vectors that contain the genes encoding *Bacillus* pantothenate and/or isoleucine-valine biosynthetic enzymes (*e.g.*, *B. subtilis* pantothenate and/or isoleucine-valine biosynthetic enzymes) are also described.

Also featured are methods of producing β-alanine that include culturing an aspartate-α-decarboxylase-overexpressing (AαD-O) microorganism under conditions such that β-alanine is produced and methods of producing β-alanine that involve contacting a composition comprising aspartate with an isolated *Bacillus* aspartate-α-
5   decarboxylase enzyme under conditions such that β-alanine is produced.

The production methods of the present invention further can include recovering the panto-compound (*e.g.*, pantothenate or pantoate).

The present invention further features recombinant microorganisms (*e.g.*, AαD-O microorganisms, microorganisms having a deregulated isoleucine-valine (*ilv*)
10   pathway, microorganisms overexpressing at least one of ketopantoate hydroxymethyltransferase (the *panB* gene product), pantothenate synthetase (the *panC* gene product), aspartate-α-decarboxylase (the *panD* gene product), ketopantoate reductase (the *panE1* gene product) and microorganisms having a deregulated *panBCD* operon. Also featured are *panB*, *panC*, *panD*, *panE*, *ilvB*, *ilvN*, *alsS*, *ilvC*, and/or *ilvD*
15   nucleic acid molecules, as well as vectors including such nucleic acid molecules and gene products encoded by such nucleic acid molecules.

The methodology of the present invention further includes, for example in addition to overexpressing at least one pantothenate biosynthetic enzyme, deleting or mutating a second pantothenate biosynthetic enzyme, said second pantothenate
20   biosynthetic enzyme preferably being downstream of the desired product in the pantothenate biosynthetic pathway. For example, mutating *panC*, in addition to overexpressing the *panE* gene product, results in even further enhanced or increased production of pantoate. Accordingly, in one embodiment, the invention features a method of producing pantoate which includes culturing a microorganism which
25   overexpresses the *panE* gene product and which has a deletion in the *panC* gene. In another embodiment, the invention features a method of producing pantoate which includes culturing a microorganism which overexpresses the *panE* gene product and/or *panB* gene product and which has a deletion in the *panC* gene. Other exemplary embodiments include a method of producing ketopantoate which includes culturing a
30   microorganism which overexpresses the *panB* gene product and which has a deletion in the *panE* gene and a method of producing β-alanine which includes culturing a microorganism which overexpresses the *panD* gene product and which has a deletion in the *panC* gene. Also included are methods of producing panto-compounds which include overexpressing at least one valine biosynthetic enzyme in a microorganism
35   which has at least one pantothenate biosynthetic enzyme deleted.

The present invention is also based at least in part, on the identification and characterization of a previously unidentified microbial pantothenate kinase gene,

coaX. *CoaX* was first identified in *Bacillus subtilis* and corresponds to an open reading frame in a portion of the chromosomal DNA that includes the 5' end of the *ftsH* gene, and all of the *yacB, yacC, yacD, cysK* and *pabB* genes. The present inventors have demonstrated that the *yacB* open reading frame encodes a novel pantothenate kinase

5   activity, the gene being unrelated by homology to any previously known pantothenate kinase gene. The gene has been renamed *coaX*, as it encodes the enzyme which catalyzes the first step in the pathway from pantothenate to CoaA.

Accordingly, the present invention features new and improved methods of producing pantothenate and other key compounds of the pantothenate biosynthetic

10   pathway (*e.g.*, panto-compounds) utilizing microorganisms having modified pantothenate kinase activity. In particular, the present invention features recombinant microorganisms that contain the *coaX* gene or that contain a mutant *coaX* gene, having reduced pantothenate kinase activity. In one embodiment, the invention features such recombinant microorganisms further having a deregulated pantothenate biosynthetic

15   pathway. In another embodiment, the invention features such recombinant microorganisms further having a deregulated isoleucine-valine (*ilv*) pathway. In a preferred embodiment, the microorganisms belong to the genus *Bacillus* (*e.g.*, *B. subtilis*).

The present invention also features recombinant microorganisms (*e.g.*,

20   microorganisms belonging to the genus *Bacillus*, for example, *B. subtilis*) that contain the *coaA* gene or that contain a mutant *coaA* gene, optionally including a *coaX* gene or mutant thereof, having reduced pantothenate kinase activity. In one embodiment, the invention features such recombinant microorganisms further having a deregulated pantothenate biosynthetic pathway or having a deregulated isoleucine-valine (*ilv*)

25   pathway.

Also featured are vectors that contain isolated *coaX* or *coaA* genes as well as mutant *coaX* and/or *coaA* genes. Isolated nucleic acid molecules that contain isolated *coaX* genes or mutant *coaX* genes are featured in addition to isolated CoaX proteins and mutant CoaX proteins.

30   The nucleic acids, vectors and recombinant microorganisms described above are particularly useful in the methodologies of the present invention. In particular, the invention features methods of enhancing panto-compound production (*e.g.*, ketopantoate, pantoate and or pantothenate production) that include culturing a recombinant microorganism having a mutant *coaX* gene under conditions such that

35   panto-compound production is enhanced. In one embodiment, the recombinant microorganism further includes a mutant *coaA* gene. In another embodiment, the recombinant microorganism further includes a mutant *avtA* and/or mutant *ilvE* gene

- 9 -

and/or mutant *ansB* gene and/or mutant *alsD* gene. Also featured are methods for identifying pantothenate modulators utilizing the recombinant microorganisms and purified CoaX proteins of the present invention.

5   Other features and advantages of the invention will be apparent from the following detailed description and claims.

Brief Description of the Drawings

Figure 1 is a schematic representation of the pantothenate biosynthetic

10   pathway.

Figure 2 is a schematic representation of the plasmid pAN240, containing sequences ligated upstream of the $P_{26}panBCD$ cassette, equivalent to the integrated version in strain PA221.

Figure 3A is a schematic representation of the plasmid pAN004,

15   containing the *panBCD* operon expressed from P_{26} and RBS1.

Figure 3B is a schematic representation of the plasmid pAN006, containing the *panBCD* operon expressed from P_{26} and RBS2.

Figure 4 is a schematic representation of the plasmid pAN236, containing an integratable and amplifiable P_{26}-RBS2-*panE1* expression cassette.

20   *Figure 5* is a schematic representation of the construction of plasmid pAN423.

Figure 6 is a schematic representation of the construction of plasmids pAN426 and pAN427.

Figure 7 is a schematic representation of the construction of plasmids

25   pAN428 and pAN429.

Figure 8 is a schematic representation of the construction of plasmid pAN431.

Figure 9 is a schematic representation of the construction of plasmid pAN441.

30   *Figure 10* is a schematic representation of the construction of plasmid pAN440.

Figure 11 is a schematic representation of the plasmid pAN251 designed to integrate a single copy of a P_{26}-*panE1* cassette at the *panE1* locus by double crossover.

35   *Figure 12* is a schematic representation of the plasmid pAN267 designed to integrate a single copy of a P_{26}-*ilvBNC* cassette at the *amyE* locus.

Figure 13 is a schematic representation of the plasmid pAN257, a clone of *Bacillus subtilis ilvD* in a low copy vector.

Figure 14 is a schematic representation of the plasmid pAN263, designed to integrate a single copy of a P_{26}-*ilvD* cassette at the *ilvD* locus.

Figure 15 is a schematic representation of the plasmid pAN261, designed to disrupt the *Bacillus subtilis ilvD* gene with the *cat* gene.

Figure 16 is a schematic representation of the Coenzyme A biosynthetic pathway in *E. coli*.

Figure 17 is a schematic representation of the structure of pAN296, a plasmid designed to delete most of the *B. subtilis coaA* gene and substitute a chloramphenicol resistance gene.

Figure 18 is a schematic representation of the structure of the *Bacillus subtilis* genome in the region of the *coaA* gene. The scale is in base pairs and the significant open reading frames are shown by open arrows.

Figure 19 is a schematic representation of the plasmid pAN281, a plasmid for expressing *Bacillus subtilis coaA* after integration at the *bpr* locus.

Figure 20A-B depicts a multiple sequence alignment (MSA) of the amino acid sequences encoded by six known or predicted microbial *coaA* genes. SEQ ID NOs:4-6 and 1-3 correspond to the amino acid sequences of *Mycobacterium leprae* (SwissProt™ Accession No. Q9X795), *Mycobacterium tuberculosis* (SwissProt™ Accession No. O53440), *Streptomyces coelicolor* (SwissProt™ Accession No. O86799), *Haemophilus influenzae* (SwissProt™ Accession No. P44793), *Escherichia coli* SwissProt™ Accession No. P15044) and *Bacillus subtilis* (SwissProt™ Accession No. P54556), respectively. The alignment was generated using ClustalW MSA software at the GenomeNet CLUSTALW Server at the Institute for Chemical Research, Kyoto University. The following parameters were used: Pairwise Alignment, K-tuple (word) size = 1, Window size = 5, Gap Penalty = 3, Number of Top Diagonals = 5, Scoring Method = Percent; Multiple Alignment, Gap Open Penalty = 10, Gap Extension Penalty = 0.0, Weight Transition = No, Hydrophilic residues = Gly, Pro, Ser, Asn, Asp, Gln, Glu, Arg and Lys, Hydrophobic Gaps = Yes; and Scoring Matrix = BLOSUM.

Figure 21 is a schematic representation of the structure of the *Bacillus subtilis* genome in the region of the *coaX* (*yacB*) gene. The scale is in base pairs, the significant open reading frames are shown by open arrows and certain predicted restriction fragments are indicated by thick bars.

Figure 22 is a schematic representation of the structure of pAN341 and pAN342, two independent PCR-derived clones of *B. subtilis yacB* (remaned herein as *coaX*).

  Figure 23A-D depicts a multiple sequence alignment (MSA) of the amino acid sequences encoded by fourteen known or predicted microbial *coaX* genes. SEQ ID NOs:9, 74, 7-8, 75, 11, 10 and 12-18 correspond to the amino acid sequences of *Bacillus subtilis* (SwissProt™ Accession No. P37564), *Clostridium acetobulyticum* (WIT™

5 Accession No. RCA03301, Argonne National Laboratories), *Streptomyces coelicolor* (PIR™ Accession No. T36391), *Mycobacterium tuberculosis* (SwissProt™ Accession No. O06282), *Rhodobacter capsulatus* (WIT™ Accession No. RRC02473), *Desulfovibrio vulgaris* (DBJ™ Accession No. BAA21476.1), *Deinococcus radiodurans* (SwissProt™ Accession No. Q9RX54), *Thermotoga maritima* (GenBank™ Accession

10 No. AAD35964.1), *Treponema pallidum* (SwissProt™ Accession No. O83446), *Borrelia burgdorferi* (SwissProt™ Accession No.O51477), *Aquifex aeolicus* (SwissProt™ Accession No. O67753), *Synechocystis sp.* (SwissProt™ Accession No. P74045), *Helicobacter pylori* (SwissProt™ Accession No. O25533), and *Bordetella pertussis* (SwissProt™ Accession No. Q45338), respectively. The alignment was

15 generated using ClustalW MSA software at the GenomeNet CLUSTALW Server at the Institute for Chemical Research, Kyoto University. The following parameters were used: Pairwise Alignment, K-tuple (word) size = 1, Window size = 5, Gap Penalty = 3, Number of Top Diagonals = 5, Scoring Method = Percent; Multiple Alignment, Gap Open Penalty = 10, Gap Extension Penalty = 0.0, Weight Transition = No, Hydrophilic

20 residues = Gly, Pro, Ser, Asn, Asp, Gln, Glu, Arg and Lys, Hydrophobic Gaps = Yes; and Scoring Matrix = BLOSUM.

  Figure 24 depicts a multiple sequence alignment of a portion of the protein sequences of the *coaA* gene products from the following microorganisms: *Bacillus subtilis*, *Escherichia coli*, *Haemophilus influenzae*, *Mycobacterium leprae*,

25 *Mycobacterium tuberculosis*, and *Streptomyces coelicolor*. The residues that are mutated in *E. coli coaA15(Ts)* and *B. subtilis coaA282A* are indicated below and above the alignment, respectively. The portions correspond to amino acid residues 168-187 of SEQ ID NO:3, 167-186 of SEQ ID NO:2, 165-184 of SEQ ID NO:1, 169-188 of SEQ ID NO:4, 169-188 of SEQ ID NO:5 and 179-198 of SEQ ID NO:6, respectively.

30 *Figure 25* is a schematic representation of the structure of pAN294, a plasmid for integrating mutagenized *B. subtilis coaA* at its native locus.

  Figure 26 is a schematic representation of the structure of pAN336, a plasmid designed to delete *B. subtilis coaX* from its chromosomal locus and replace it with a kanamycin resistence gene.

35

Detailed Description of the Invention

The present invention features new and improved methods of producing pantothenate and other key compounds of the pantothenate biosynthetic pathway (referred to herein as "panto-compounds", for example, pantothenate, ketopantoate, pantoate and β-alanine) using microorganisms in which the pantothenate biosynthetic pathway has been manipulated such that pantothenate or other desired panto-compounds are produced.

The new and improved methodologies of the present invention include methods of producing panto-compounds (*e.g.*, pantothenate) in microorganisms having at least one enzyme of the pantothenate biosynthetic pathway manipulated such that pantothenate or other desired panto-compounds are produced (*e.g.*, produced at an increased level). For example, the invention features methods of producing panto-compounds (*e.g.*, pantothenate) in microorganisms having at least one of ketopantoate hydroxymethyltransferase, ketopantoate reductase, pantothenate synthetase or aspartate-α-decarboxylase manipulated such that pantothenate or other desired panto-compounds are produced. The methodologies of the present invention also include methods of producing panto-compounds (*e.g.*, pantothenate) in microorganisms having at least one valine-isoleucine biosynthetic enzyme, described herein, manipulated such that pantothenate or other desired panto-compounds are produced. For example, the invention features methods of producing panto-compounds (*e.g.*, pantothenate) in microorganisms having at least one of acetohydroxyacid synthetase, acetohydroxyacid isomeroreductase or dihydroxyacid dehydratase manipulated such that pantothenate or other desired panto-compounds are produced.

The invention also features methods of producing panto-compounds that involve culturing a ketopantoate reductase-overexpressing (KPAR-O) microorganism under conditions such that the panto-compound is produced. The invention also features methods of producing pantothenate in a manner independent of precursor feed that involve culturing an aspartate-α-decarboxylase-overexpressing (AαD-O) microorganism under conditions such that pantothenate is produced. Also featured are β-alanine independent high yield pantothenate production methods as well as methods of producing β-alanine. The present invention also features methods for enhancing production of panto-compounds that involve culturing pantothenate kinase mutants. In particular, the present invention features new and improved methods of producing pantothenate and other key compounds of the pantothenate biosynthetic pathway (*e.g.*, panto-compounds) utilizing microorganisms having modified pantothenate kinase activity, for example, microorganisms that include the *coaX* gene or that include a mutant *coaX* gene, having reduced pantothenate kinase activity.

In order that the present invention may be more readily understood, certain terms are first defined herein.

The term "pantothenate biosynthetic pathway" includes the biosynthetic pathway involving pantothenate biosynthetic enzymes (*e.g.*, polypeptides encoded by biosynthetic enzyme-encoding genes), compounds (*e.g.*, precursors, substrates, intermediates or products), cofactors and the like utilized in the formation or synthesis of pantothenate. The term "pantothenate biosynthetic pathway" includes the biosynthetic pathway leading to the synthesis of pantothenate in a microorganisms (*e.g.*, *in vivo*) as well as the biosynthetic pathway leading to the synthesis of pantothenate *in vitro*. Figure 1 includes a schematic representation of the pantothenate biosynthetic pathway. Pantothenate biosynthetic enzymes are depicted in bold and their corresponding genes indicated in italics.

The term "pantothenate biosynthetic enzyme" includes any enzyme utilized in the formation of a compound (*e.g.*, intermediate or product) of the pantothenate biosynthetic pathway. According to Figure 1, synthesis of pantoate from α-ketoisovalerate (α-KIV) proceeds *via* the intermediate, ketopantoate. Formation of ketopantoate is catalyzed by the pantothenate biosynthetic enzyme ketopantoate hydroxymethyltransferase (the *panB* gene product). Formation of pantoate is catalyzed by the pantothenate biosynthetic enzyme ketopantoate reductase (the *panE* gene product). Synthesis of β-alanine from aspartate is catalyzed by the pantothenate biosynthetic enzyme aspartate-α-decarboxylase (the *panD* gene product). Formation of pantothenate from pantoate and β-alanine (*e.g.*, condensation) is catalyzed by the pantothenate biosynthetic enzyme pantothenate synthetase (the *panC* gene product).

The term "isoleucine-valine biosynthetic pathway" includes the biosynthetic pathway involving isoleucine-valine biosynthetic enzymes (*e.g.*, polypeptides encoded by biosynthetic enzyme-encoding genes), compounds (*e.g.*, precursors, substrates, intermediates or products), cofactors and the like utilized in the formation or synthesis of conversion of pyruvate to valine or isoleucine. The term "isoleucine-valine biosynthetic pathway" includes the biosynthetic pathway leading to the synthesis of valine or isoleucine in a microorganisms (*e.g.*, *in vivo*) as well as the biosynthetic pathway leading to the synthesis of valine or isoleucine *in vitro*. Figure 1 includes a schematic representation of the isoleucine-valine biosynthetic pathway. Isoleucine-valine biosynthetic enzymes are depicted in bold italics and their corresponding genes indicated in italics

The term "isoleucine-valine biosynthetic enzyme" includes any enzyme utilized in the formation of a compound (*e.g.*, intermediate or product) of the isoleucine-valine biosynthetic pathway. According to Figure 1, synthesis of valine from pyruvate

proceeds *via* the intermediates, acetolactate, α,β-dihydroxyisovalerate (α,β-DHIV) and α-ketoisovalerate (α-KIV). Formation of acetolactate from pyruvate is catalyzed by the isoleucine-valine biosynthetic enzyme acetohydroxyacid synthetase (the *ilvBN* gene product, or alternatively, the *alsS* gene product). Formation of α,β-DHIV from

5 acetolactate is catalyzed by the isoleucine-valine biosynthetic enzyme acetohydroxyacidisomero reductase (the *ilvC* gene product). Synthesis of α-KIV from α,β-DHIV is catalyzed by the isoleucine-valine biosynthetic enzyme dihydroxyacid dehydratase (the *ilvD* gene product). Moreover, valine and isoleucine can be interconverted by branched chain amino acid transaminases.

10 As used herein, each of ketopantoate, pantoate, β-alanine and pantothenate are "panto-compounds". The term "panto-compound" includes a compound (*e.g.*, a substrate, intermediate or product) in the pantothenate biosynthetic pathway which is downstream from a particular pantothenate biosynthetic enzyme. In one example, a panto-compound is downstream of the pantothenate biosynthetic enzyme

15 ketopantoate hydroxymethyltransferase (the *panB* gene product) and can include ketopantoate, pantoate and/or pantothenate. In another example, a panto-compound is downstream of the pantothenate biosynthetic enzyme ketopantoate reductase (the *panE* gene product) and can include pantoate and/or pantothenate. In yet another example, a panto-compound is downstream of the pantothenate biosynthetic enzyme pantothenate

20 synthetase (the *panC* gene product) and can include pantothenate. In yet another example, a panto-compound is downstream of the pantothenate biosynthetic enzyme aspartate-α-decarboxylase (the *panD* gene product) and can include β-alanine and/or pantothenate.

Preferred panto-compounds include pantothenate and pantoate. The term

25 "pantothenate" includes the free acid form of pantothenate, also referred to as "pantothenic acid" as well as any salt thereof (*e.g.*, derived by replacing the acidic hydrogen of pantothenate or pantothenic acid with a cation, for example, calcium, sodium, potassium, ammonium), also referred to as a "pantothenate salt". The term "panto-compound" also includes alcohol derivatives of pantothenate. Preferred

30 pantothenate salts are calcium pantothenate or sodium pantothenate. A preferred alcohol derivative is pantothenol. Pantothenate salts and/or alcohols of the present invention include salts and/or alcohols prepared *via* conventional methods from the free acids described herein. In another embodiment, calcium pantothenate is synthesized directly by a microorganism of the present invention. A pantothenate salt of the present

35 invention can likewise be converted to a free acid form of pantothenate or pantothenic acid by conventional methodology.

The term "pantoate" includes the free acid form of pantoate, also referred to as "pantoic acid" as well as any salt thereof (*e.g.*, derived by replacing the acidic hydrogen of pantoate or pantoic acid with a cation, for example, calcium, sodium, potassium, ammonium), also referred to as a "pantoate salt". Preferred pantoate salts are

5   calcium pantoate or sodium pantoate. Pantoate salts of the present invention include salts prepared *via* conventional methods from the free acids described herein. A pantoate salt of the present invention can likewise be converted to a free acid form of pantoate or pantoic acid by conventional methodology. Moreover, a free acid form of pantoate or pantoic acid can be converted to pantolactone by conventional methodology.

10   The term "CoA biosynthetic pathway" includes the biosynthetic pathway involving CoA biosynthetic enzymes (*e.g.*, polypeptides encoded by biosynthetic enzyme-encoding genes), compounds (*e.g.*, precursors, substrates, intermediates or products), cofactors and the like utilized in the formation or synthesis of CoA from pantothenate. A schematic representation of the CoA biosynthetic pathway in *E. coli* is

15   set forth as Figure 16. (The pathway depicted is also presumed to be that utilized by other microorganisms.) The term "CoA biosynthetic pathway" includes the biosynthetic pathway leading to the synthesis of CoA in microorganisms (*e.g.*, *in vivo*) as well as the biosynthetic pathway leading to the synthesis of CoA *in vitro*. The term "Coenzyme A or CoA biosynthetic enzyme" includes any enzyme utilized in the formation of a

20   compound (*e.g.*, intermediate or product) of the CoA biosynthetic pathway, for example, the *coaA*, *panK* or *coaX* gene product which catalyzes the phosphorylation of pantothenate to form 4'-phosphopantothenate, or the *coaD* gene product which catalyzes the conversion of 4'-phosphopantetheine to dephosphocoenzyme A.

25

I.   *Recombinant Microorganisms and Methods for Culturing Microorganisms Such That Panto-Compounds are Produced*

The methodologies of the present invention feature microorganisms, *e.g.*, recombinant microorganisms, preferably including vectors or genes (*e.g.*, wild-type

30   and/or mutated genes) as described herein and/or cultured in a manner which results in the production of a desired product (*e.g.* a panto-compound or panto-compounds). The term "recombinant" microorganism includes a microorganism (*e.g.*, bacteria, yeast cell, fungal cell, etc.) which has been genetically altered, modified or engineered (*e.g.*, genetically engineered) such that it exhibits an altered, modified or different genotype

35   and/or phenotype (*e.g.*, when the genetic modification affects coding nucleic acid sequences of the microorganism) as compared to the naturally-occurring microorganism from which it was derived. Preferably, a "recombinant" microorganism of the present

invention has been genetically engineered such that it overexpresses at least one bacterial gene or gene product (*e.g.*, a pantothenate or isoleucine-valine biosynthetic enzyme encoding-gene) as described herein, preferably a biosynthetic enzyme encoding-gene included within a recombinant vector as described herein and/or a biosynthetic

5   enzyme expressed from a recombinant vector. The ordinary skilled will appreciate that a microorganism expressing or overexpressing a gene product produces or overproduces the gene product as a result of expression or overexpression of nucleic acid sequences and/or genes encoding the gene product.

The term "manipulated microorganism" includes a microorganism that

10   has been engineered (*e.g.*, genetically engineered) or modified such that the microorganism has at least one enzyme of the pantothenate biosynthetic pathway and/or at least one enzyme of the isoleucine-valine biosynthetic pathway modified such that pantothenate or other desired panto-compounds are produced. Modification or engineering of such microorganisms can be according to any methodology described

15   herein including, but not limited to, deregulation of a biosynthetic pathway and/or overexpression of at least one biosynthetic enzyme. A "manipulated" enzyme (*e.g.*, a "manipulated" biosynthetic enzyme) includes an enzyme, the expression or production of which has been altered or modified such that at least one upstream or downstream precursor, substrate or product of the enzyme is altered or modified, for example, as

20   compared to a corresponding wild-type or naturally occurring enzyme.

The term "overexpressed" or "overexpression" includes expression of a gene product (*e.g.*, a pantothenate biosynthetic enzyme or isoleucine-valine biosynthetic enzyme) at a level greater than that expressed prior to manipulation of the microorganism or in a comparable microorganism which has not been manipulated. In

25   one embodiment, the microorganism can be genetically manipulated (*e.g.*, genetically engineered) to overexpress a level of gene product greater than that expressed prior to manipulation of the microorganism or in a comparable microorganism which has not been manipulated. Genetic manipulation can include, but is not limited to, altering or modifying regulatory sequences or sites associated with expression of a particular gene

30   (*e.g.*, by adding strong promoters, inducible promoters or multiple promoters or by removing regulatory sequences such that expression is constitutive), modifying the chromosomal location of a particular gene, altering nucleic acid sequences adjacent to a particular gene such as a ribosome binding site or transcription terminator, increasing the copy number of a particular gene, modifying proteins (*e.g.*, regulatory proteins,

35   suppressors, enhancers, transcriptional activators and the like) involved in transcription of a particular gene and/or translation of a particular gene product, or any other conventional means of deregulating expression of a particular gene routine in the art

(including but not limited to use of antisense nucleic acid molecules, for example, to block expression of repressor proteins).

In another embodiment, the microorganism can be physically or environmentally manipulated to overexpress a level of gene product greater than that expressed prior to manipulation of the microorganism or in a comparable microorganism which has not been manipulated. For example, a microorganism can be treated with or cultured in the presence of an agent known or suspected to increase transcription of a particular gene and/or translation of a particular gene product such that transcription and/or translation are enhanced or increased. Alternatively, a microorganism can be cultured at a temperature selected to increase transcription of a particular gene and/or translation of a particular gene product such that transcription and/or translation are enhanced or increased.

The term "deregulated" or "deregulation" includes the alteration or modification of at least one gene in a microorganism that encodes an enzyme in a biosynthetic pathway, such that the level or activity of the biosynthetic enzyme in the microorganism is altered or modified. Preferably, at least one gene that encodes an enzyme in a biosynthetic pathway is altered or modified such that the gene product is enhanced or increased. The phrase "deregulated pathway" can also include a biosynthetic pathway in which more than one gene that encodes an enzyme in a biosynthetic pathway is altered or modified such that the level or activity of more than one biosynthetic enzyme is altered or modified. The ability to "deregulate" a pathway (e.g., to simultaneously deregulate more than one gene in a given biosynthetic pathway) in a microorganism arises from the particular phenomenon of microorganisms in which more than one enzyme (e.g., two or three biosynthetic enzymes) are encoded by genes occurring adjacent to one another on a contiguous piece of genetic material termed an "operon".

The term "operon" includes a coordinated unit of gene expression that contains a promoter and possibly a regulatory element associated with one or more, preferably at least two, structural genes (e.g., genes encoding enzymes, for example, biosynthetic enzymes). Expression of the structural genes can be coordinately regulated, for example, by regulatory proteins binding to the regulatory element or by anti-termination of transcription. The structural genes can be transcribed to give a single mRNA that encodes all of the structural proteins. Due to the coordinated regulation of genes included in an operon, alteration or modification of the single promoter and/or regulatory element can result in alteration or modification of each gene product encoded by the operon. Alteration or modification of the regulatory element can include, but is not limited to removing the endogenous promoter and/or regulatory element(s), adding

strong promoters, inducible promoters or multiple promoters or removing regulatory sequences such that expression of the gene products is modified, modifying the chromosomal location of the operon, altering nucleic acid sequences adjacent to the operon or within the operon such as a ribosome binding site, increasing the copy number

5 of the operon, modifying proteins (*e.g.*, regulatory proteins, suppressors, enhancers, transcriptional activators and the like) involved in transcription of the operon and/or translation of the gene products of the operon, or any other conventional means of deregulating expression of genes routine in the art (including but not limited to use of antisense nucleic acid molecules, for example, to block expression of repressor

10 proteins). Deregulation can also involve altering the coding region of one or more genes to yield, for example, an enzyme that is feedback resistant or has a higher or lower specific activity.

A particularly preferred "recombinant" microorganism of the present invention has been genetically engineered to overexpress a bacterially-derived gene or

15 gene product. The term "bacterially-derived" or "derived-from", for example bacteria, includes a gene which is naturally found in bacteria or a gene product (*e.g.*, ketopantoate hydroxymethyltransferase, ketopantoate reductase, pantothenate synthetase, aspartate-α-decarboxylate, acetohydroxyacid synthetase, acetohydroxyacid isomeroreductase or dihydroxyacid dehydratase) which is encoded by a bacterial gene (*e.g.*, encoded by

20 *panB, panE, panC, panD, ilvB, ilvN, alsS, ilvC,* or *ilvD*).

The methodologies of the present invention feature recombinant microorganisms which overexpress at least one of ketopantoate hydroxymethyltransferase, ketopantoate reductase, pantothenate synthetase or aspartate-α-decarboxylase. A particularly preferred recombinant microorganism of the present

25 invention has been genetically engineered to overexpress a *Bacillus* (*e.g., Bacillus licheniformis, Bacillus amyloliquefaciens, Bacillus halodurans, Bacillus subtilis,* and *Bacillus pumilus,* etc.) biosynthetic enzyme (*e.g.,* has been engineered to overexpress at least one of *B. subtilis* ketopantoate reductase (the *panE* gene product) (*e.g.,* ketopantoate reductase having the amino acid sequence of SEQ ID NO:30 or encoded by

30 the nucleic acid sequence of SEQ ID NO:29), *B. subtilis* ketopantoate hydroxymethyltransferase (the *panB* gene product) (*e.g.,* ketopantoate hydroxymethyltransferase having the amino acid sequence of SEQ ID NO:24 or encoded by a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:23), *B. subtilis* pantothenate synthetase (the *panC* gene product) (*e.g.,* pantothenate

35 synthetase having the amino acid sequence of SEQ ID NO:26 or encoded by a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:25) and/or *B. subtilis* aspartate-α-decarboxylase (the *panD* gene product) (*e.g.,* aspartate-α-decarboxylase

having the amino acid sequence of SEQ ID NO:28 or encoded by a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:27).

In an exemplary embodiment, the invention features a microorganism (*e.g.*, a KPAR-O microorganism) that has been transformed with a vector comprising a *panE* nucleic acid sequence (*e.g.*, a *panE* nucleic acid seqeunce as set forth in SEQ ID NO:29). In another embodiment, the invention features a microorganism that has been transformed with a vector comprising a *panB* nucleic acid sequence (*e.g.*, a *panB* nucleic acid sequence as set forth in SEQ ID NO:23), a vector comprising a *panC* nucleic acid sequence (*e.g.*, a *panC* nucleic acid sequence as set forth in SEQ ID NO:25) or a vector comprising a *panD* nucleic acid sequence (*e.g.*, a *panD* nucleic acid sequence as set forth in SEQ ID NO:27). In yet another embodiment, the invention features a microorganism having a deregulated *panBCD* operon (*e.g.*, SEQ ID NO:59).

Other preferred "recombinant" microorganisms of the present invention have a deregulated isoleucine-valine (*ilv*) pathway. The phrase "microorganism having a deregulated isoleucine-valine (*ilv*) pathway" includes a microorganism having an alteration or modification in at least one gene encoding an enzyme of the isoleucine-valine (*ilv*) pathway or having an alteration or modification in an operon including more than one gene encoding an enzyme of the isoleucine-valine (*ilv*) pathway. A preferred "microorganism having a deregulated isoleucine-valine (*ilv*) pathway" has been genetically engineered to overexpress a *Bacillus* (*e.g.*, *B. subtilis*) *ilv* biosynthetic enzyme (*e.g.*, has been engineered to overexpress at least one of acetohydroxyacid synthetase (the *ilvBN* gene products or the *alsS* gene product) (*e.g.*, acetohydroxyacid synthetase having subunits having the amino acid sequences of SEQ ID NO:32 and SEQ ID NO:34 or encoded by nucleic acid molecules having the nucleotide sequence of SEQ ID NO:31 and SEQ ID NO:33 or the nucleotide sequence of SEQ ID NO:58 from nucleotides 1-2246 or acetohydroxyacid synthetase encoded by a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:86), acetohydroxyacid isomeroreductase (the *ilvC* gene product) (*e.g.*, acetohydroxyacid isomeroreductase having the amino acid sequence of SEQ ID NO:36 or encoded by a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:35), dihydroxyacid dehydratase (the *ilvD* gene product) (*e.g.*, dihydroxyacid dehydratase having the amino acid sequence of SEQ ID NO:38 or encoded by a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:37), and/or has been transformed with a vector comprising an *ilvBNC* nucleic acid sequence (SEQ ID NO:58, coding regions from nucleotides 1-1725, 1722-2246 and 2263-3291) and/or an *ilvD* nucleic acid sequence (SEQ ID NO:37).

In another preferred embodiment, a recombinant microorganism is designed or engineered such that a mutant CoaA and/or CoaX biosynthetic enzyme is

expressed and at least one pantothenate biosynthetic enzyme and/or at least one isoleucine-valine biosynthetic enzyme is overexpressed or deregulated.

In another preferred embodiment, a microorganism of the present invention overexpresses or is mutated for a gene or biosynthetic enzyme (*e.g.*, a CoA
5   biosynthetic enzyme, pantothenate biosynthetic enzyme or isoleucine-valine biosynthetic enzyme) which is bacterially-derived. The term "bacterially-derived" or "derived-from", for example bacteria, includes a gene product (*e.g.*, ketopantoate hydroxymethyltransferase, ketopantoate reductase, pantothenate synthetase, aspartate-α-decarboxylate, acetohydroxyacid synthetase, acetohydroxyacid isomeroreductase,
10   dihydroxyacid dehydratase or pantothenate kinase) which is encoded by a bacterial gene (*e.g.*, *panB*, *panE*, *panC*, *panD*, *ilvBN* (or *alsS*), *ilvC*, *ilvD*, or encoded by *coaA* or *coaX*).

Still other preferred recombinant microorganisms of the present invention are mutant microorganisms. As used herein, the term "mutant microorganism" includes
15   a recombinant microorganism that has been genetically engineered to express a mutated gene or protein that is normally or naturally expressed by the microorganism. Preferably, a mutant microorganism expresses a mutated gene or protein such that the microorganism exhibits an altered, modified or different phenotype (*e.g.*, has been engineered to express a mutated CoaA biosynthetic enzyme, for example, pantothenate
20   kinase). In one embodiment, a mutant microorganism is designed or engineered such that it includes a mutant *coaX* gene, as defined herein. In another embodiment, a recombinant microorganism is designed or engineered such that it includes a mutant *coaA* gene, as defined herein. In another embodiment, a mutant microorganism is designed or engineered such that a *coaX* gene has been deleted (*i.e.*, the protein encoded
25   by the *coaX* gene is not produced). In another embodiment, a mutant microorganism is designed or engineered such that a *coaA* gene has been deleted (*i.e.*, the protein encoded by the *coaA* gene is not produced). Preferably, a mutant microorganism has a mutant *coaX* gene or a mutant *coaA* gene, or has been engineered to have a *coaX* gene and/or *coaA* deleted, such that that the mutant microorganism encodes a "reduced pantothenate
30   kinase activity". In the context of a whole microorganism, a "reduced pantothenate kinase activity" can be determined by measuring or assaying for a decrease in an intermediate or product of the CoA biosynthetic pathway, for example, measuring or assaying for 4'-phosphopantothenate, 4'-phosphopantothenylcysteine, 4'-phosphopantetheine, dephosphocoenzyme A, Coenzyme A, apo-acyl carrier protein
35   (apo-ACP) or holo-acyl carrier protein (ACP) in the microorganism (*e.g.*, in a lysate isolated or derived from the microorganism) or in the medium in which the microorganism is cultured (see *e.g.*, Figure 16). Alternatively, a "reduced pantothenate

kinase activity" can be determined by measuring or assaying for decreased growth of the microorganism. Alternatively, a "reduced pantothenate kinase activity" can be determined by measuring or assaying for an increase in a panto-compound (*e.g.*, pantothenate) in the microorganism or surrounding media, as panto-compounds lie

5 upstream of the CoA biosynthetic pathway, the first step of which is catalyzed by pantothenate kinase. The invention also features recombinant microorganisms that, in addition to having reduced pantothenate kinase activity (*e.g.*, expressing mutant *coaA* and/or mutant *coaX* genes) have a deregulated pantothenate biosynthesis pathway and/or a deregulated isoleucine-valine (*ilv*) biosynthetic pathway.

10 In one embodiment, a recombinant microorganism of the present invention is a Gram positive organism (*e.g.*, a microorganism which retains basic dye, for example, crystal violet, due to the presence of a Gram-positive wall surrounding the microorganism). In a preferred embodiment, the recombinant microorganism is a microorganism belonging to a genus selected from the group consisting of *Bacillus*,

15 *Cornyebacterium, Lactobacillus, Lactococci* and *Streptomyces*. In a more preferred embodiment, the recombinant microorganism is of the genus *Bacillus*. In another preferred embodiment, the recombinant microorganism is selected from the group consisting of *Bacillus subtilis, Bacillus lentimorbus, Bacillus lentus, Bacillus firmus, Bacillus pantothenticus, Bacillus amyloliquefaciens, Bacillus cereus, Bacillus circulans,*

20 *Bacillus coagulans, Bacillus licheniformis, Bacillus megaterium, Bacillus pumilus, Bacillus thuringiensis*, and other Group 1 *Bacillus* species, for example, as characterized by 16S rRNA type (Priest (1993) in *Bacillus subtilis and Other Gram-Positive Bacteria* eds. Sonenshein *et al.*, ASM, Washington, D.C., p. 6). In another preferred embodiment, the recombinant microorganism is *Bacillus brevis* or *Bacillus*

25 *stearothermophilus*. In another preferred embodiment, the recombinant microorganism is selected from the group consisting of *Bacillus licheniformis, Bacillus amyloliquefaciens, Bacillus halodurans, Bacillus subtilis*, and *Bacillus pumilus*.

In another embodiment, the recombinant microorganism is a Gram negative (excludes basic dye) organism. In a preferred embodiment, the recombinant

30 microorganism is a microorganism belonging to a genus selected from the group consisting of *Salmonella, Escherichia, Klebsiella, Serratia,* and *Proteus*. In a more preferred embodiment, the recombinant microorganism is of the genus *Escherichia*. In an even more preferred embodiment, the recombinant microorganism is *Escherichia coli*. In another embodiment, the recombinant microorganism is *Saccharomyces* (*e.g.*, *S.*

35 *cerevisiae*).

An important aspect of the present invention involves culturing the recombinant microorganisms described herein, such that a desired compound (*e.g.*, a

desired panto-compound) is produced. The term "culturing" includes maintaining and/or growing a living microorganism of the present invention (*e.g.*, maintaining and/or growing a culture or strain). In one embodiment, a microorganism of the invention is cultured in liquid media. In another embodiment, a microorganism of the invention is

5   cultured in solid media or semi-solid media. In a preferred embodiment, a microorganism of the invention is cultured in media (*e.g.*, a sterile, liquid media) comprising nutrients essential or beneficial to the maintenance and/or growth of the microorganism (*e.g.*, carbon sources or carbon substrate, for example complex carbohydrates such as bean or grain meal, starches, sugars, sugar alcohols,

10   hydrocarbons, oils, fats, fatty acids, organic acids and alcohols; nitrogen sources, for example, vegetable proteins, peptones, peptides and amino acids derived from grains, beans and tubers, proteins, peptides and amino acids derived form animal sources such as meat, milk and animal byproducts such as peptones, meat extracts and casein hydrolysates; inorganic nitrogen sources such as urea, ammonium sulfate, ammonium

15   chloride, ammonium nitrate and ammonium phosphate; phosphorus sources, for example, phosphoric acid, sodium and potassium salts thereof; trace elements, for example, magnesium, iron, manganese, calcium, copper, zinc, boron, molybdenum, and/or cobalt salts; as well as growth factors such as amino acids, vitamins, growth promoters and the like).

20   Preferably, microorganisms of the present invention are cultured under controlled pH. The term "controlled pH" includes any pH which results in production of the desired product (*e.g.*, a panto-compound). In one embodiment, microorganisms are cultured at a pH of about 7. In another embodiment, microorganisms are cultured at a pH of between 6.0 and 8.5. The desired pH may be maintained by any number of

25   methods known to those skilled in the art.

             Also preferably, microorganisms of the present invention are cultured under controlled aeration. The term "controlled aeration" includes sufficient aeration (*e.g.*, oxygen) to result in production of the desired product (*e.g.*, panto-compound). In one embodiment, aeration is controlled by regulating oxygen levels in the culture, for

30   example, by regulating the amount of oxygen dissolved in culture media. Preferably, aeration of the culture is controlled by agitating the culture. Agitation may be provided by a propeller or similar mechanical agitation equipment, by revolving or shaking the growth vessel (*e.g.*, fermentor) or by various pumping equipment. Aeration may be further controlled by the passage of sterile air or oxygen through the medium (e.g.,

35   through the fermentation mixture). Also preferably, microorganisms of the present invention are cultured without excess foaming (*e.g.*, *via* addition of antifoaming agents).

Moreover, microorganisms of the present invention can be cultured under controlled temperatures. The term "controlled temperature" includes any temperature which results in production of the desired product (e.g., a panto-compound). In one embodiment, controlled temperatures include temperatures between 15°C and 95°C. In another embodiment, controlled temperatures include temperatures between 15°C and 70°C. Preferred temperatures are between 20°C and 55°C, more preferably between 30°C and 45°C or between 30°C and 50°C.

Microorganisms can be cultured (e.g., maintained and/or grown) in liquid media and preferably are cultured, either continuously or intermittently, by conventional culturing methods such as standing culture, test tube culture, shaking culture (e.g., rotary shaking culture, shake flask culture, etc.), aeration spinner culture, or fermentation. In a preferred embodiment, the microorganisms are cultured in shake flasks. In a more preferred embodiment, the microorganisms are cultured in a fermentor (e.g., a fermentation process). Fermentation processes of the present invention include, but are not limited to, batch, fed-batch and continuous methods of fermentation. The phrase "batch process" or "batch fermentation" refers to a closed system in which the composition of media, nutrients, supplemental additives and the like is set at the beginning of the fermentation and not subject to alteration during the fermentation, however, attempts may be made to control such factors as pH and oxygen concentration to prevent excess media acidification and/or microorganism death. The phrase "fed-batch process" or "fed-batch" fermentation refers to a batch fermentation with the exception that one or more substrates or supplements are added (e.g., added in increments or continuously) as the fermentation progresses. The phrase "continuous process" or "continuous fermentation" refers to a system in which a defined fermentation media is added continuously to a fermentor and an equal amount of used or "conditioned" media is simultaneously removed, preferably for recovery of the desired product (e.g., panto-compound). A variety of such processes have been developed and are well-known in the art.

The phrase "culturing under conditions such that a desired compound (e.g., a panto-compound, for example, pantothenate) is produced" includes maintaining and/or growing microorganisms under conditions (e.g., temperature, pressure, pH, duration, etc.) appropriate or sufficient to obtain production of the desired compound or to obtain desired yields of the particular compound being produced. For example, culturing is continued for a time sufficient to produce the desired amount of a panto-compound (e.g., pantothenate, pantoate or β-alanine). Preferably, culturing is continued for a time sufficient to substantially reach maximal production of the panto-compound. In one embodiment, culturing is continued for about 12 to 24 hours. In another

embodiment, culturing is continued for about 24 to 36 hours, 36 to 48 hours, 48 to 72 hours, 72 to 96 hours, 96 to 120 hours, 120 to 144 hours, or greater than 144 hours. In another embodiment, culturing is continued for a time sufficient to reach production yields of panto-compound, for example, cells are cultured such that at least about 15 to 20 g/L of panto-compound are produced, at least about 20 to 25 g/L panto-compound are produced, at least about 25 to 30 g/L panto-compound are produced, at least about 30 to 35 g/L panto-compound are produced, at least about 35 to 40 g/L panto-compound are produced (e.g., at least about 37 g/L panto-compound) or at least about 40 to 50 g/L panto compound are produced. In yet another embodiment, microorganisms are cultured under conditions such that a preferred yield of panto-compound, for example, a yield within a range set forth above, is produced in about 24 hours, in about 36 hours, in about 48 hours, in about 72 hours, or in about 96 hours.

The methodology of the present invention can further include a step of recovering a desired compound (e.g., a panto-compound). The term "recovering" a desired compound (e.g., a panto-compound) includes extracting, harvesting, isolating or purifying the compound from culture media. Recovering the compound can be performed according to any conventional isolation or purification methodology known in the art including, but not limited to, treatment with a conventional resin (e.g., anion or cation exchange resin, non-ionic adsorption resin, etc.), treatment with a conventional adsorbent (e.g., activated charcoal, silicic acid, silica gel, cellulose, alumina, etc.), alteration of pH, solvent extraction (e.g., with a conventional solvent such as an alcohol, ethyl acetate, hexane and the like), dialysis, filtration, concentration, crystallization, recrystallization, pH adjustment, lyophilization and the like. For example, a compound (e.g., a panto-compound) can be recovered from culture media by first removing the microorganisms from the culture. Media is then passed through or over a cation exchange resin to remove unwanted cations and then through or over an anion exchange resin to remove unwanted inorganic anions and organic acids having stronger acidities than the panto-compound of interest (e.g., pantothenate). The resulting panto-compound (e.g., pantothenate) can subsequently be converted to a pantothenate salt (e.g., calcium pantothenate) as described herein.

Preferably, a desired compound of the present invention is "extracted", "isolated" or "purified" such that the resulting preparation is substantially free of other components (e.g., free of media components and/or fermentation byproducts). The language "substantially free of other components" includes preparations of desired compound in which the compound is separated (e.g., purified or partially purified) from media components or fermentation byproducts of the culture from which it is produced. In one embodiment, the preparation has greater than about 80% (by dry weight) of the

desired compound (*e.g.*, less than about 20% of other media components or fermentation byproducts), more preferably greater than about 90% of the desired compound (*e.g.*, less than about 10% of other media components or fermentation byproducts), still more preferably greater than about 95% of the desired compound (*e.g.*, less than about 5% of

5   other media components or fermentation byproducts), and most preferably greater than about 98-99% desired compound (*e.g.*, less than about 1-2% other media components or fermentation byproducts). When the desired compound is a panto-compound that has been derivatized to a salt (*e.g.* a pantothenate salt or pantoate salt), the panto-compound is preferably further free (*e.g.*, substantially free) of chemical contaminants associated

10   with the formation of the salt. When the desired compound is a panto-compound that has been derivatized to an alcohol, the panto-compound is preferably further free (*e.g.*, substantially free) of chemical contaminants associated with the formation of the alcohol.

           In an alternative embodiment, the desired panto-compound is not purified

15   from the microorganism, for example, when the microorganism is biologically non-hazardous (*e.g.*, safe). For example, the entire culture (or culture supernatant) can be used as a source of product (*e.g.*, crude product). In one embodiment, the culture (or culture supernatant) supernatant is used without modification. In another embodiment, the culture (or culture supernatant) is concentrated. In yet another embodiment, the

20   culture (or culture supernatant) is dried or lyophilized.

           II.   Panto-Compound Production Methodologies Featuring
Ketopantoate Reductase-Overexpressing Microorganisms

25   One aspect of the invention features methods of producing a panto-compounds that involve culturing a ketopantoate reductase-overexpressing (KPAR-O) microorganism under conditions such that the panto-compound is produced. The term "ketopantoate reductase-overexpressing (KPAR-O) microorganism" includes a microorganism which has been manipulated such that ketopantoate reductase is

30   overexpressed (*e.g.*, a *B. subtilis* ketopantoate reductase protein having the amino acid sequence of SEQ ID NO:30) and/or has been transformed with a vector comprising a *panE1* nucleic acid sequence (*e.g.*, a *B. subtilis panE1* nucleic acid sequence as set forth in SEQ ID NO:29). In one embodiment, the panto-compound is pantothenate. In another embodiment, the panto-compound is pantoate. In another embodiment, the

35   ketopantoate reductase is bacterial-derived. In another embodiemnt, the ketopantoate reductase is derived from *Bacillus* (*e.g.*, is derived from *Bacillus subtilis*). In yet another embodiment, the KPAR-O microorganism is Gram positive. In yet another

embodiment, the KPAR-O microorganism is a microorganism belonging to a genus selected from the group consisting of *Bacillus, Cornyebacterium, Lactobacillus, Lactococci* and *Streptomyces*. In a preferred embodiemnt, the KPAR-O microorganism is of the genus *Bacillus*. In a more preferred embodiment, the KPAR-O microorganism

5   is selected from the group consisting of *Bacillus licheniformis, Bacillus amyloliquefaciens, Bacillus halodurans, Bacillus subtilis* and *Bacillus pumilus*. In a particularly preferred embodiemnt, the KPAR-O microorganism is *Bacillus subtilis*.

In still other embodiments, the KPAR-O microorganism further overexpresses at least one pantothenate biosynthetic enzyme in addition to ketopantoate

10   reductase. In an exemplary embodiment, the KPAR-O microorganism further overexpresses at least one of ketopantoate hydroxymethyltransferase, pantothenate synthetase and aspartate-α-decarboxylase. Also featured are methods of producing panto-compounds, for example, methods that involve culturing a KPAR-O microorganism, which further include the step of recovering the panto-compound.

15

III.   Methods of Producing Panto-Compounds Independent of Precursor Feed Requirements

Depending on the biosynthetic enzyme or combination of biosynthetic enzymes manipulated, it may be desirable or necessary to provide (*e.g.*, feed)

20   microorganisms of the present invention at least one pantothenate biosynthetic precursor such that pantothenate or other desired panto-compounds are produced. The term "pantothenate biosynthetic precursor" or "precursor" includes an agent or compound which, when provided to, brought into contact with, or included in the culture medium of a microorganism, serves to enhance or increase pantothenate biosynthesis. In one

25   embodiment, the pantothenate biosynthetic precursor or precursor is aspartate. In another embodiment, the pantothenate biosynthetic precursor or precursor is β-alanine. The amount of aspartate or β-alanine added is preferably an amount that results in a concentration in the culture medium sufficient to enhance productivity of the microorganism (*e.g.*, a concentration sufficient to enhance production of a panto-

30   compound, for example, β-alanine, ketopantoate, pantoate or pantothenate). Pantothenate biosynthetic precursors of the present invention can be added in the form of a concentrated solution or suspension (*e.g.*, in a suitable solvent such as water or buffer) or in the form of a solid (*e.g.*, in the form of a powder). Moreover, pantothenate biosynthetic precursors of the present invention can be added as a single aliquot,

35   continuously or intermittently over a given period of time.

In yet another embodiment, the pantothenate biosynthetic precursor is valine, see *e.g.*, Example III. In yet another embodiment, the pantothenate biosynthetic

precursor is α-ketoisovalerate. Preferably, valine or α-ketoisovalerate is added in an amount that results in a concentration in the medium sufficient for production of the desired product (*e.g.*, panto-compound) to occur. Pantothenate biosynthetic precursors are also referred to herein as "supplemental pantothenate biosynthetic substrates".

5   Providing pantothenate biosynthetic precursors in the pantothenate biosynthetic methodologies of the present invention, can be associated with high costs, for example, when the methodologies are used to produce high yields of panto-compounds. Accordingly, preferred methodologies of the present invention feature microorganisms having at least one biosynthetic enzyme or combination of biosynthetic

10   enzymes (*e.g.*, at least one pantothenate biosynthetic enzyme and/or valine-isoleucine biosynthetic enzyme) manipulated such that pantothenate or other desired panto-compounds are produced in a manner independent of precursor feed. The phrase "a manner independent of precursor feed", for example, when referring to a method for producing a desired compound (*e.g.*, a panto-compound), includes an approach to or a

15   mode of producing the desired compound that does not depend or rely on precursors being provided (*e.g.*, fed) to the microorganism being utilized to produce the desired compound. For example, microorganisms featured in the methodologies of the present invention can be used to produce panto-compounds in a manner requiring no feeding of the precursors aspartate, β-alanine, valine and/or α-KIV.

20   Alternative preferred methodologies of the present invention feature microorganisms having at least one biosynthetic enzyme or combination of biosynthetic enzymes manipulated such that pantothenate or other desired panto-compounds are produced in a manner substantially independent of precursor feed. The phrase "a manner substantially independent of precursor feed" includes an approach to or a

25   method of producing the desired compound that depends or relies to a lesser extent on precursors being provided (*e.g.*, fed) to the microorganism being utilized. For example, microorganisms featured in the methodologies of the present invention can be used to produce panto-compounds in a manner requiring feeding of substantially reduced amounts of the precursors aspartate, β-alanine, valine and/or α-KIV. In one

30   embodiment, the invention features methods of producing panto-compounds (*e.g.*, pantothenate) in a manner that requires feeding of less than 5%-10% of the amount of precursor required by a control microorganism (*e.g.*, a microorganism that is dependent, for example is wholly dependent, on precursor feed to efficiently produce the desired compound). In another embodiment, the invention features methods of producing

35   panto-compounds in a manner that requires feeding of less than 15-20% of the amount of precursor required by a control microorganism. In another embodiment, the invention features methods of producing panto-compounds in a manner that requires

feeding of less than 25-30%, 35-40%, 45-50% or 55-60% of the amount of precursor required by a control microorganism. As described in Examples I-III herein, particular microorganisms featured in the methodologies of the present invention require, for example, 5 g/L of aspartate, β-alanine, valine or α-KIV (*e.g.*, in test tube or in shake

5 flask cultures). Accordingly, in a preferred embodiment, the present invention features methods of producing panto-compounds (*e.g.*, pantothenate) in a manner requiring feeding of less than 0.25 g/L, 0.5 g/L, 0.75 g/L, 1 g/L, 1.25 g/L, 1.5 g/L, 1.75 g/L, 2 g/L, 2.25 g/L, 2.5 g/L, 2.75 g/L or 3 g/L.

Preferred methods of producing desired compounds (*e.g.*, panto-

10 compounds) in a manner independent of precursor feed or alternatively, in a manner substantially independent of precursor feed, involve culturing microorganisms which have been manipulated (*e.g.*, designed or engineered, for example, genetically engineered) such that expression of at least one pantothenate biosynthetic enzyme, and/or at least one isoleucine-valine biosynthetic enzyme, is modified. For example, in

15 one embodiment, a microorganism is manipulated (*e.g.*, designed or engineered) such that the production of at least one pantothenate biosynthetic enzyme, and/or at least one isoleucine/valine biosynthetic enzyme is deregulated. In a preferred embodiment, a microorganism is manipulated (*e.g.*, designed or engineered) such that it has a deregulated biosynthetic pathway, for example, a deregulated pantothenate biosynthesis

20 pathway and/or a deregulated isoleucine-valine biosynthetic pathway, as defined herein. In another preferred embodiment, a microorganism is manipulated (*e.g.*, designed or engineered) such that at least one pantothenate biosynthetic enzyme, and/or at least one isoleucine-valine biosynthetic enzyme is overexpressed.

Preferred methods of producing desired compounds (*e.g.*, panto-

25 compounds) in a manner independent of precursor feed or alternatively, in a manner substantially independent of precursor feed, are as follows. In one embodiment, the invention features a method of producing pantothenate in a manner independent of precursor feed comprising culturing an aspartate-α-decarboxylase-overexpressing (AαD-O) microorganism having a deregulated isoleucine-valine (*ilv*) pathway under conditions

30 such that pantothenate is produced. In another embodiment, the invention features a method of producing pantothenate in a manner independent of precursor feed comprising culturing an aspartate-α-decarboxylase-overexpressing (AαD-O) microorganism having a deregulated pantothenate (*pan*) pathway and a deregulated isoleucine-valine (*ilv*) pathway, under conditions such that pantothenate is produced. In

35 another embodiment, the invention features a method of producing pantothenate in a manner independent of aspartate or β-alanine feed comprising culturing an aspartate-α-decarboxylase-overexpressing (AαD-O) microorganism under conditions such that

pantothenate is produced. In yet another embodiment, the invention features a method of producing pantothenate in a manner independent of valine or α-ketoisovalerate feed comprising culturing a microorganism having a deregulated isoleucine-valine (*ilv*) biosynthetic pathway under conditions such that pantothenate is produced.

5   The term "aspartate-α-decarboxylase-overexpressing (AαD-O) microorganism" includes a microorganism which has been manipulated such that aspartate-α-decarboxylase is overexpressed. A preferred "aspartate-α-decarboxylase-overexpressing (AαD-O) microorganism" has been transformed with a vector comprising a *B. subtilis panD* nucleic acid sequence (*e.g.*, a *panD* nucleic acid sequence

10 that encodes an aspartate-α-decarboxylase protein having the amino acid sequence of SEQ ID NO:28, for example, a *panD* nucleic acid sequence as set forth in SEQ ID NO:27).

           The phrase "microorganism having a deregulated isoleucine-valine (*ilv*) pathway" includes a microorganism having an alteration or modification in at least one

15 gene encoding an enzyme of the isoleucine-valine (*ilv*) pathway or having an alteration or modification in an operon including more than one gene encoding an enzyme of the isoleucine-valine (*ilv*) pathway. A preferred "microorganism having a deregulated isoleucine-valine (*ilv*) pathway" overexpresses acetohydroxyacid synthetase (*e.g.*, acetohydroxyacid synthetase having subunits having the amino acid sequences of SEQ

20 ID NO:32 and SEQ ID NO:34 or acetohydroxyacid synthetase having the amino acid sequence of SEQ ID NO:87), acetohydroxyacid isomeroreductase (having the amino acid sequence of SEQ ID NO:36), or dihydroxyacid dehydratase (having the amino acid sequence of SEQ ID NO:38) and/or has been transformed with a vector comprising *ilvB*, *ilvN*, *ilvC*, *ilvBN*, *ilvBNC* or *alsS* nucleic acid sequences (SEQ ID NO:31, SEQ ID

25 NO:33, SEQ ID NO:35, nucleotides 1-2246 of SEQ ID NO:58, SEQ ID NO:58 having coding regions from nucleotides 1-1725, 1722-2246 and 2263-3291, or SEQ ID NO:86, respectively) and/or an *ilvD* nucleic acid sequence (SEQ ID NO:37).

IV.   High Yield Production Methodologies

30   A particularly preferred embodiment of the present invention is a high yield production method for producing pantothenate comprising culturing a manipulated microorganism under conditions such that pantothenate is produced at a significantly high yield. The phrase "high yield production method", for example, a high yield production method for producing a desired compound (*e.g.*, for producing a panto-

35 compound) includes a method that results in production of the desired compound at a level which is elevated or above what is usual for comparable production methods. Preferably, a high yield production method results in production of the desired

compound at a significantly high yield. The phrase "significantly high yield" includes a level of production or yield which is sufficiently elevated or above what is usual for comparable production methods, for example, which is elevated to a level sufficient for commercial production of the desired product (*e.g.*, production of the product at a

5   commercially feasible cost). In one embodiment, the invention features a high yield production method of producing pantothenate that includes culturing a manipulated microorganism under conditions such that pantothenate is produced at a level greater than 2 g/L. In another embodiment, the invention features a high yield production method of producing pantothenate that includes culturing a manipulated microorganism

10   under conditions such that pantothenate is produced at a level greater than 10 g/L. In another embodiment, the invention features a high yield production method of producing pantothenate that includes culturing a manipulated microorganism under conditions such that pantothenate is produced at a level greater than 20 g/L. In yet another embodiment, the invention features a high yield production method of

15   producing pantothenate that includes culturing a manipulated microorganism under conditions such that pantothenate is produced at a level greater than 30 g/L. In yet another embodiment, the invention features a high yield production method of producing pantothenate that includes culturing a manipulated microorganism under conditions such that pantothenate is produced at a level greater than 40 g/L.

20   The invention further features a high yield production method for producing a desired compound (*e.g.*, for producing a panto-compound) that involves culturing a manipulated microorganism under conditions such that a sufficiently elevated level of compound is produced within a commercially desireable period of time. In an exemplary embodiment, the invention features a high yield production

25   method of producing pantothenate that includes culturing a manipulated microorganism under conditions such that pantothenate is produced at a level greater than 15-20 g/L in 36 hours. In another embodiment, the invention features a high yield production method of producing pantothenate that includes culturing a manipulated microorganism under conditions such that pantothenate is produced at a level greater than 25-30 g/L in 48

30   hours. In another embodiment, the invention features a high yield production method of producing pantothenate that includes culturing a manipulated microorganism under conditions such that pantothenate is produced at a level greater than 35-40 g/L in 72 hours, for example, greater that 37 g/L in 72 hours. In another embodiment, the invention features a high yield production method of producing pantothenate that

35   includes culturing a manipulated microorganism under conditions such that pantothenate is produced at a level greater than 30-40 g/L in 60 hours, for example, greater that 30, 35 or 40 g/L in 60 hours. Values and ranges included and/or intermediate within the ranges

set forth herein are also intended to be within the scope of the present invention. For example, pantothenate production at levels of at least 31, 32, 33, 34, 35, 36, 37, 38 and 39 g/L in 60 hours are intended to be included within the range of 30-40 g/L in 60 hours. In another example, ranges of 30-35 g/L or 35-40 g/L are intended to be included within the range of 30-40 g/L in 60 hours. Moreover, the skilled artisan will appreciate that culturing a manipulated microorganism to achieve a production level of, for example, "30-40 g/L in 60 hours" includes culturing the microorganism for additional time periods (*e.g.*, time periods longer than 60 hours), optionally resulting in even higher yields of pantothenate being produced.

V. Panto-Compound Production Methodologies Featuring Pantothenate Kinase Mutant Microorganisms

The present invention relates to methods of producing pantothenate using microorganisms engineered to produce high yields of pantothenate as well as other panto-compounds. Cells overproducing pantothenate result in high intracellular pantothenate levels that could overcome the feedback inhibition of pantothenate kinase by CoA, leading to overproduction of CoA. Besides consuming pantothenate, increased synthesis of CoA may cause increased feedback inhibition of the PanB, PanD, PanE or PanC reaction, thereby limiting pantothenate production. Accordingly, a reduction in pantothenate kinase activity may lead to a decrease in CoA levels with resulting increases in PanB, PanD, PanE or PanC activity and pantothenate production.

Thus, certain methodologies of the present invention are based, at least in part, on the identification and characterization of the *B. subtilis coaA* gene and the demonstration that the gene is neither essential for *B. subtilis* growth (*i.e.*, deletion of the *coaA* gene from the chromosome of *B. subtilis* is not a lethal event) nor for pantothenate kinase activity in *B. subtilis*. A second pantothenate kinase-encoding gene has been identified and characterized in *B. subtilis*, and is termed "*coaX*". This gene complements an *E. coli* mutant that contains a temperature sensitive pantothenate kinase and is not related by homology to any previously known pantothenate kinase gene.

In one aspect, the methodologies of the invention feature recombinant microorganisms that include the *coaX* gene or that include a mutant *coaX* gene, having reduced pantothenate kinase activity. In one embodiment, the methodologies feature such recombinant microorganisms further having a deregulated pantothenate biosynthetic pathway. In another embodiment, the methodologies feature such recombinant microorganisms further having a deregulated isoleucine-valine (*ilv*) pathway. In a preferred embodiment, the microorganisms belong to the genus *Bacillus* (*e.g.*, *B. subtilis*).

The methodologies of the invention also feature recombinant microorganisms (*e.g.*, microorganisms belong to the genus *Bacillus*, for example, *B. subtilis*) that include the *coaA* gene or that include a mutant *coaA* gene, optionally including a *coaX* gene or mutant thereof, having reduced pantothenate kinase activity.

5 In one embodiment, the methodologies feature such recombinant microorganisms further having a deregulated pantothenate biosynthetic pathway or having a deregulated isoleucine-valine (*ilv*) pathway. Also featured are vectors that include isolated *coaX* or *coaA* genes as well as mutant *coaX* and/or *coaA* genes. Isolated nucleic acid molecules that include isolated *coaX* genes or mutant *coaX* genes are features in addition to

10 isolated CoaX proteins and mutant CoaX proteins.

The above-described nucleic acid molecules (*e.g.*, genes), proteins, vectors, and recombinant microorganisms (*e.g.*, mutant microorganisms), are particularly suited for use in methods of producing panto-compounds and/or methods of enhancing panto-compound production. In one embodiment, the invention features a

15 method for producing a panto-compound (*e.g.*, pantothenate) that includes culturing a pantothenate kinase mutant (*e.g.*, a recombinant microorganism that misexpresses, *e.g.*, is mutated for, pantothenate kinase, as defined herein) under conditions such that panto-compound is produced. In another embodiment, the invention features a method for enhancing production of a panto-compound (*e.g.*, pantothenate) that includes culturing a

20 pantothenate kinase mutant (*e.g.*, a recombinant microorganism that misexpresses, *e.g.*, is mutated for, pantothenate kinase, as defined herein) under conditions such that production of the panto-compound is produced. As used herein, the term "enhancing" (for example, in the context of the phrase "enhancing production") includes increasing the level or rate of production of panto-compound (*e.g.*, pantothenate) as compared to

25 the level or rate of production in a non-mutant microorganism (*e.g.*, a microorganism having a normal pantothenate kinase gene(s) and/or having normal pantothenate production rates and/or levels.

Preferably, the level of panto-compound produced in methodologies featuring the pantothenate kinase mutants of the present invention is increased by at

30 least 5% as compared to the level produced by a non-mutant (*e.g.*, a recombinant microorganism expressing non-mutated pantothenate kinase). Even more preferably, the level of panto-compound is increased 10% as compared to methodologies featuring non-mutants. Even more preferably, panto-compound levels (*e.g.*, pantothenate levels) are increased 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, are increased 2-fold, 5-fold, 10-

35 fold, 50-fold, 100-fold or more as compared to methodologies featuring non-mutants.

VI.   *Additional Mutations Resulting in Enhanced Panto-Compound Production*

The methodologies of the present invention further can include, for example in addition to overexpressing or deregulating a pantothenate biosynthetic enzyme and/or an isoleucine-valine biosynthetic enzyme, or in addition to mutating a pantothenate-kinase encoding gene, deleting or mutating an enzyme that catalyzes the conversion of key pantothenate biosynthesis substrates or precursors to unwanted or undesirable products. For example, mutating the *ilvE* gene (Kuramitsu *et al.* (1985) *J. Biochem.* 97:993-999) or a homologue thereof (SEQ ID NO:62 or SEQ ID NO:64), thereby limiting the conversion of α-ketoisovalerate to valine, in addition to mutating a pantothenate kinase encoding enzyme, is predicted to result in even further enhanced or increased production of panto-compound. Alternatively, mutating the *ansB* gene (Sun and Seflow (1991) *J. Bacteriol.* 173:3831-3845) or a homologue thereof (SEQ ID NO:66), thereby limiting the degradation of aspartate, in addition to mutating a pantothenate kinase encoding enzyme, is predicted to result in even further enhanced or increased production of panto-compound. Alternatively, mutating the *alsD* gene (Renna *et al.* (1993) *J. Bacteriol.* 175:3863-3875) or a homologue thereof (SEQ ID NO:68), thereby limiting the conversion of acetolactate to acetoin, in addition to mutating a pantothenate kinase encoding enzyme, is predicted to result in even further enhanced or increased production of panto-compound. Alternatively, mutating the *avtA* gene encoding alanine-valine transaminase or a homologue thereof, thereby limiting the conversion of α-ketoisovalerate to valine, in addition to mutating a pantothenate kinase encoding enzyme, is predicted to result in even further enhanced or increased production of panto-compound. Mutating the *avtA* gene can include mutating, for example, an *avtA* gene having the nucleotide sequence of SEQ ID NO:70 (*e.g.*, the *E. coli avtA* gene), or a structural homolog thereof (*e.g.*, a homologue encoding a protein having 30-40%, 40-50%, 50-60%, 60-70%, 70-80%, 80-90%, 90-95% or more identity with the amino acid sequence of SEQ ID NO:71) or a functional homologue (*e.g.*, a gene encoding a structurally unrelated protein having alanine-valine transmainase activity. Such mutations can be accomplished using the methodologies as exemplified in the Examples (*e.g.*, Examples XIII, XV, XVI and XVII).

Accordingly, in one embodiment, the invention features a method of producing a panto-compound which includes culturing a microorganism having a mutant pantothenate kinase-encoding gene and which further has a deletion or mutation in an *avtA*, *ilvE*, *ansB*, and/or *alsD* gene, or homologue thereof. In another embodiment, the invention features a method of producing a panto-compound which includes

culturing a microorganism having a mutant pantothenate-kinase encoding gene and a deregulated pantothenate biosynthetic pathway enzyme and which further has a deletion or mutation in an *avtA, ilvE, ansB*, and/or *alsD* gene, or homologue thereof. In another embodiment, the invention features a method of producing a panto-compound which

5   includes culturing a microorganism having a mutant pantothenate-kinase encoding gene and a deregulated isoleucine-valine biosynthetic pathway enzyme and which further has a deletion or mutation in an *avtA, ilvE, ansB*, and/or *alsD* gene, or homologue thereof.

Mutating the *alsD* gene can be particularly useful when accomplished in conjunction with overexpression or deregulation of the *alsS* gene, for example, to

10   prevent carbon (*e.g.*, acetolactate) from being drawn away from the precursor pool utilized for α-KIV production. Accordingly, to maximize the contribution of the *als* locus to panto-compound production, it is desirable to disrupt the *alsD* gene in addition to overexpressing the *alsS* gene. To disrupt the *alsD* gene, appropriate fragments of the *als* operon, flanking the *alsD* gene, are amplified by PCR and cloned to provide

15   homology for creating the disruptions. A drug resistance gene, such as the *cat* gene, is cloned between the flanking DNA fragments in place of the *alsD* gene, and the linearized DNA is transformed into a pantothenate production strain such as PA824, selecting for drug-resistance. To overexpress *alsS,* the *alsS* coding sequence (*e.g.*, an *alsS* coding sequence that has been engineered by PCR for expression) is cloned into an

20   expression vector. Vectors which express *alsS* (or alternatively, vectors which express *alsS* plus *ilvC*) are the introduced into panto-compound production strains (*e.g.*, the pantothenate producing strain PA824).

The methodologies of the present invention further can include, for example in addition to overexpressing or deregulating a pantothenate biosynthetic

25   enzyme and/or an isoleucine-valine biosynthetic enzyme, or in addition to mutating a pantothenate-kinase encoding gene, deleting or mutating an enzyme that catalyzes the conversion of desired panto-compounds to unwanted or undesireable downstream products.

30   ### VII.   Isolated Nucleic Acid Molecules and Genes

Another aspect of the present invention features isolated nucleic acid molecules that encode *Bacillus* proteins (*e.g.*, *B. subtilis* proteins), for example, *Bacillus* pantothenate biosynthetic enzymes (*e.g.*, *B. subtilis* pantothenate biosynthetic enzymes) or *Bacillus* valine-isoleucine biosynthetic enzymes (*e.g.*, *B. subtilis* valine-isoleucine

35   biosynthetic enzymes). Also featured are isolated *coaX* and/or *coaA* nucleic acid molecules (*e.g.*, isolated *coaX* and/or *coaA* genes) as well as isolated nucleic acid molecules that include such *coaX* and/or *coaA* nucleic acid molecules or genes.

The term "nucleic acid molecule" includes DNA molecules (*e.g.*, linear, circular, cDNA or chromosomal DNA) and RNA molecules (*e.g.*, tRNA, rRNA, mRNA) and analogs of the DNA or RNA generated using nucleotide analogs. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is

5 double-stranded DNA. The term "isolated" nucleic acid molecule includes a nucleic acid molecule which is free of sequences which naturally flank the nucleic acid molecule (*i.e.*, sequences located at the 5' and 3' ends of the nucleic acid molecule) in the chromosomal DNA of the organism from which the nucleic acid is derived. In various embodiments, an isolated nucleic acid molecule can contain less than about 10

10 kb, 5 kb, 4kb, 3kb, 2kb, 1 kb, 0.5 kb, 0.1 kb, 50 bp, 25 bp or 10 bp of nucleotide sequences which naturally flank the nucleic acid molecule in chromosomal DNA of the microorganism from which the nucleic acid molecule is derived. Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular materials when produced by recombinant techniques, or substantially free

15 of chemical precursors or other chemicals when chemically synthesized.

The term "gene", as used herein, includes a nucleic acid molecule (*e.g.*, a DNA molecule or segment thereof), for example, a protein or RNA-encoding nucleic acid molecule, that in an organism, is separated from another gene or other genes, by intergenic DNA (*i.e.*, intervening or spacer DNA which naturally flanks the gene and/or

20 separates genes in the chromosomal DNA of the organism). A gene may direct synthesis of an enzyme or other protein molecule (*e.g.*, may comprise coding sequences, for example, a contiguous open reading frame (ORF) which encodes a protein) or may itself be functional in the organism. A gene in an organism, may be clustered in an operon, as defined herein, said operon being separated from other genes and/or operons

25 by the intergenic DNA. Individual genes contained within an operon may overlap without intergenic DNA between said individual genes. An "isolated gene", as used herein, includes a gene which is essentially free of sequences which naturally flank the gene in the chromosomal DNA of the organism from which the gene is derived (*i.e.*, is free of adjacent coding sequences which encode a second or distinct protein or RNA

30 molecule, adjacent structural sequences or the like) and optionally includes 5' and 3' regulatory sequences, for example promoter sequences and/or terminator sequences. In one embodiment, an isolated gene includes predominantly coding sequences for a protein (*e.g.*, sequences which encode *Bacillus* proteins). In another embodiment, an isolated gene includes coding sequences for a protein (*e.g.*, for a *Bacillus* protein) and

35 adjacent 5' and/or 3' regulatory sequences from the chromosomal DNA of the organism from which the gene is derived (*e.g.*, adjacent 5' and/or 3' *Bacillus* regulatory sequences). Preferably, an isolated gene contains less than about 10 kb, 5 kb, 2 kb, 1 kb,

0.5 kb, 0.2 kb, 0.1 kb, 50 bp, 25 bp or 10 bp of nucleotide sequences which naturally flank the gene in the chromosomal DNA of the organism from which the gene is derived.

In one aspect, the present invention features isolated *panB* nucleic acid sequences or genes, isolated *panC* nucleic acid sequences or genes, isolated *panD* nucleic acid sequences or genes, isolated *panE* nucleic acid sequences or genes, isolated *ilvB, ilvN, ilvBN* nucleic acid sequences or genes, isolated *alsS* nucleic acid sequences or genes, isolated *ilvC* nucleic acid sequences or genes and/or isolated *ilvD* nucleic acid sequences or genes.

In a preferred embodiment, the nucleic acid or gene is derived from *Bacillus* (*e.g.*, is *Bacillus*-derived). The term "derived from *Bacillus*" or "*Bacillus*-derived" includes a nucleic acid or gene which is naturally found in microorganisms of the genus *Bacillus*. Preferably, the nucleic acid or gene is derived from a microorganism selected from the group consisting of *Bacillus subtilis, Bacillus lentimorbus, Bacillus lentus, Bacillus firmus, Bacillus pantothenticus, Bacillus amyloliquefaciens, Bacillus cereus, Bacillus circulans, Bacillus coagulans, Bacillus licheniformis, Bacillus megaterium, Bacillus pumilus, Bacillus thuringiensis*, and other Group 1 *Bacillus* species, for example, as characterized by 16S rRNA type (Priest, *supra*). In another preferred embodiment, the nucleic acid or gene is derived from *Bacillus brevis* or *Bacillus stearothermophilus*. In another preferred embodiment, the nucleic acid molecules and/or genes of the present invention are derived from a microorganism selected from the group consisting of *Bacillus licheniformis, Bacillus amyloliquefaciens, Bacillus halodurans, Bacillus subtilis*, and *Bacillus pumilus*. In a particularly preferred embodiment, the nucleic acid or gene is derived from *Bacillus subtilis* (*e.g.*, is *Bacillus subtilis*-derived). The term "derived from *Bacillus subtilis*" or "*Bacillus subtilis*-derived" includes a nucleic acid or gene which is naturally found in *Bacillus subtilis*. In yet another preferred embodiment, the nucleic acid or gene is a *Bacillus* gene homologue (*e.g.*, is derived from a species distinct from *Bacillus* but having significant homology to a *Bacillus* gene of the present invention, for example, a *Bacillus pan* gene or *Bacillus ilv* gene).

Included within the scope of the present invention are bacterial-derived nucleic acid molecules or genes and/or *Bacillus*-derived nucleic acid molecules or genes (*e.g.*, B. *subtilis*-derived nucleic acid molecules or genes), for example, the genes identified by the present inventors, for example, *Bacillus* or *B. subtilis coaX* genes, *coaA* genes, *pan* genes and/or *ilv* genes. Further included within the scope of the present invention are bacterial-derived nucleic acid molecules or genes and/or *Bacillus*-derived nucleic acid molecules or genes (*e.g.*, *B. subtilis*-derived nucleic acid molecules or

genes) (*e.g.*, *B. subtilis* nucleic acid molecules or genes) which differ from naturally-occurring bacterial and/or *Bacillus* nucleic acid molecules or genes (*e.g.*, *B. subtilis* nucleic acid molecules or genes), for example, nucleic acid molecules or genes which have nucleic acids that are substituted, inserted or deleted, but which encode proteins

5   substantially similar to the naturally-occurring gene products of the present invention. In one embodiment, an isolated nucleic acid molecule comprises at least one of the nucleotide sequences set forth as SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO 31, SEQ ID NO:33, SEQ ID NO:86, SEQ ID NO:35 or SEQ ID NO:37. In another preferred embodiment, an isolated nucleic acid molecule comprises

10 at least two, three or four of the nucleotide sequences set forth as SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO 31, SEQ ID NO:33, SEQ ID NO:88, SEQ ID NO:35 or SEQ ID NO:37. For example, a preferred isolated nucleic acid molecule of the present invention can include the nucleotide sequences of SEQ ID NO:23, SEQ ID NO:25 and SEQ ID NO:27, preferably linked such that the proteins

15 encoded by the nucleotide sequences of SEQ ID NO:23, SEQ ID NO:25 and SEQ ID NO:27 are each produced when the isolated nucleic acid molecule is expressed in a microorganism (*e.g.*, SEQ ID NO:59). In another example, a preferred isolated nucleic acid molecule of the present invention can include the nucleotide sequences of SEQ ID NO:31 and SEQ ID NO:33, preferably linked such that the proteins encoded by the

20 nucleotide sequences of SEQ ID NO:31 and SEQ ID NO:33 are each produced when the isolated nucleic acid molecule is expressed in a microorganism (*e.g.*, nucleotides 1-2246 of SEQ ID NO:58). In another example, a preferred isolated nucleic acid molecule of the present invention can include the nucleotide sequence of SEQ ID NO:86. In another example, a preferred isolated nucleic acid molecule of the present invention can include

25 the nucleotide sequences of SEQ ID NO:31, SEQ ID NO:33 and SEQ ID NO:35, preferably linked such that the proteins encoded by the nucleotide sequences of SEQ ID NO:31, SEQ ID NO:33 and SEQ ID NO:35 are each produced when the isolated nucleic acid molecule is expressed in a microorganism (*e.g.*, SEQ ID NO:58).

In another embodiment, an isolated nucleic acid molecule of the present

30 invention comprises a nucleotide sequence which is at least about 60-65%, preferably at least about 70-75%, more preferable at least about 80-85%, and even more preferably at least about 90-95% or more identical to a nucleotide sequence set forth as SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO 31, SEQ ID NO:33, SEQ ID NO:88, SEQ ID NO:35 or SEQ ID NO:37. In another embodiment, an

35 isolated nucleic acid molecule hybridizes under stringent conditions to a nucleic acid molecule having a nucleotide sequence set forth as SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO 31, SEQ ID NO:33, SEQ ID NO:88, SEQ

ID NO:35 or SEQ ID NO:37. Such stringent conditions are known to those skilled in the art and can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. A preferred, non-limiting example of stringent (*e.g.* high stringency) hybridization conditions are hybridization in 6X sodium chloride/sodium

5   citrate (SSC) at about 45°C, followed by one or more washes in 0.2 X SSC, 0.1% SDS at 50-65°C. Preferably, an isolated nucleic acid molecule of the invention that hybridizes under stringent conditions to the sequence of SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO 31, SEQ ID NO:33, SEQ ID NO:88, SEQ ID NO:35 or SEQ ID NO:37 corresponds to a naturally-occurring nucleic

10   acid molecule. As used herein, a "naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature.

      A nucleic acid molecule of the present invention (*e.g.*, a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO 31, SEQ ID NO:33, SEQ ID NO:88, SEQ ID

15   NO:35 or SEQ ID NO:37 can be isolated using standard molecular biology techniques and the sequence information provided herein. For example, nucleic acid molecules can be isolated using standard hybridization and cloning techniques (*e.g.*, as described in Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory,* Cold Spring Harbor Laboratory Press, Cold

20   Spring Harbor, NY, 1989) or can be isolated by the polymerase chain reaction using synthetic oligonucleotide primers designed based upon the sequence of SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO 31, SEQ ID NO:33, SEQ ID NO:88, SEQ ID NO:35 or SEQ ID NO:37. A nucleic acid of the invention can be amplified using cDNA, mRNA or alternatively, genomic DNA, as a template and

25   appropriate oligonucleotide primers according to standard PCR amplification techniques. In another preferred embodiment, an isolated nucleic acid molecule of the invention comprises a nucleic acid molecule which is a complement of the nucleotide sequence shown in SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:33, SEQ ID NO 31, SEQ ID NO:33, SEQ ID NO:88, SEQ ID NO:35.

30   Additional *panC* nucleic acid sequences include those that comprise the nucleotide sequence of SEQ ID NO:25, encode a homologue of the polypeptide having the amino acid sequence set forth in SEQ ID NO:26 (*e.g.*, encode a polypeptide having at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or more identity to the polypeptide having the amino acid sequence as set forth in SEQ ID NO:26 and a substantially

35   identical activity as said polypeptide), hybridize under stringent conditions to all or a portion of a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:25 or to all or a portion of a nucleic acid molecule that encodes a polypeptide having the

amino acid sequence of SEQ ID NO:26, or are complementary to a *panC* nucleotide sequence as set forth herein.

Aditional *panD* nucleic acid sequences include those that comprise the nucleotide sequence of SEQ ID NO:27, encode a homologue of the polypeptide having

5   the amino acid sequence set forth in SEQ ID NO:28 (*e.g.*, encode a polypeptide having at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or more identity to the polypeptide having the amino acid sequence as set forth in SEQ ID NO:28 and a substantially identical activity as said polypeptide), hybridize under stringent conditions to all or a portion of a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:27 or

10   to all or a portion of a nucleic acid molecule that encodes a polypeptide having the amino acid sequence of SEQ ID NO:28, or are complementary to a *panD* nucleotide sequence as set forth herein.

Additional *panE* nucleic acid sequences include those that comprise the nucleotide sequence of SEQ ID NO:29, encode a homologue of the polypeptide having

15   the amino acid sequence set forth in SEQ ID NO:30 (*e.g.*, encode a polypeptide having at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or more identity to the polypeptide having the amino acid sequence as set forth in SEQ ID NO:30 and a substantially identical activity as said polypeptide), hybridize under stringent conditions to all or a portion of a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:29 or

20   to all or a portion of a nucleic acid molecule that encodes a polypeptide having the amino acid sequence of SEQ ID NO:30, or are complementary to a *panE* nucleotide sequence as set forth herein.

Additional *ilvB* nucleic acid sequences are those that comprise the nucleotide sequence of SEQ ID NO:31, encode a homologue of the polypeptide having

25   the amino acid sequence set forth in SEQ ID NO:32 (*e.g.*, encode a polypeptide having at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or more identity to the polypeptide having the amino acid sequence as set forth in SEQ ID NO:32 and a substantially identical activity as said polypeptide), hybridize under stringent conditions to all or a portion of a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:31 or

30   to all or a portion of a nucleic acid molecule that encodes a polypeptide having the amino acid sequence of SEQ ID NO:32, or are complementary to an *ilvB* nucleotide sequence as set forth herein.

Additional *ilvN* nucleic acid sequences are those that comprise the nucleotide sequence of SEQ ID NO:33, encode a homologue of the polypeptide having

35   the amino acid sequence set forth in SEQ ID NO:34 (*e.g.*, encode a polypeptide having at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or more identity to the polypeptide having the amino acid sequence as set forth in SEQ ID NO:34 and a substantially

identical activity as said polypeptide), hybridize under stringent conditions to all or a portion of a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:33 or to all or a portion of a nucleic acid molecule that encodes a polypeptide having the amino acid sequence of SEQ ID NO:34, or are complementary to an *ilvN* nucleotide

5   sequence as set forth herein.

Additional *ilvC* nucleic acid sequences include those that comprise the nucleotide sequence of SEQ ID NO:35, encode a homologue of the polypeptide having the amino acid sequence set forth in SEQ ID NO:36 (*e.g.*, encode a polypeptide having at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or more identity to the polypeptide

10   having the amino acid sequence as set forth in SEQ ID NO:36 and a substantially identical activity as said polypeptide), hybridize under stringent conditions to all or a portion of a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:35 or to all or a portion of a nucleic acid molecule that encodes a polypeptide having the amino acid sequence of SEQ ID NO:36, or are complementary to an *ilvC* nucleotide

15   sequence as set forth herein.

Additional *ilvD* nucleic acid sequences include those that comprise the nucleotide sequence of SEQ ID NO:37, encode a homologue of the polypeptide having the amino acid sequence set forth in SEQ ID NO:38 (*e.g.*, encode a polypeptide having at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or more identity to the polypeptide

20   having the amino acid sequence as set forth in SEQ ID NO:38 and a substantially identical activity as said polypeptide), hybridize under stringent conditions to all or a portion of a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:37 or to all or a portion of a nucleic acid molecule that encodes a polypeptide having the amino acid sequence of SEQ ID NO:38, or are complementary to an *ilvD* nucleotide

25   sequence as set forth herein.

Additional *alsS* nucleic acid sequences include those that comprise the nucleotide sequence of SEQ ID NO:86, encode a homologue of the polypeptide having the amino acid sequence set forth in SEQ ID NO:87 (*e.g.*, encode a polypeptide having at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or more identity to the polypeptide

30   having the amino acid sequence as set forth in SEQ ID NO:87 and a substantially identical activity as said polypeptide), hybridize under stringent conditions to all or a portion of a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:86 or to all or a portion of a nucleic acid molecule that encodes a polypeptide having the amino acid sequence of SEQ ID NO:87, or are complementary to an *alsS* nucleotide

35   sequence as set forth herein.

In another embodiment, an isolated nucleic acid molecule is or includes a *coaX* gene, or portion or fragment thereof. In one embodiment, an isolated *coaX* nucleic

acid molecule or gene comprises the nucleotide sequence as set forth in SEQ ID NO:19 (*e.g.*, comprises the *B. subtilis coaX* nucleotide sequence). In another embodiment, an isolated *coaX* nucleic acid molecule or gene comprises a nucleotide sequence that encodes the amino acid sequence as set forth in SEQ ID NO:9 (*e.g.*, encodes the *B.*

5   *subtilis* CoaX amino acid sequence). In yet another embodiment, an isolated *coaX* nucleic acid molecule or gene encodes a homologue of the CoaX protein having the amino acid sequence of SEQ ID NO:9. As used herein, the term "homologue" includes a protein or polypeptide sharing at least about 30-35%, preferably at least about 35-40%, more preferably at least about 40-50%, and even more preferably at least about 60%,

10 70%, 80%, 90% or more identity with the amino acid sequence of a wild-type protein or polypeptide described herein and having a substantially equivalent functional or biological activity as said wild-type protein or polypeptide. For example, a CoaX homologue shares at least about 30-35%, preferably at least about 35-40%, more preferably at least about 40-50%, and even more preferably at least about 60%, 70%,

15 80%, 90% or more identity with the protein having the amino acid sequence set forth as SEQ ID NO:9 and has a substantially equivalent functional or biological activity (*i.e.*, is a functional equivalent) of the protein having the amino acid sequence set forth as SEQ ID NO:9 (*e.g.*, has a substantially equivalent pantothenate kinase activity). In a preferred embodiment, an isolated *coaX* nucleic acid molecule or gene comprises a

20 nucleotide sequence that encodes a polypeptide as set forth in any one of SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:74 or SEQ ID NO:75. In another embodiment, an isolated *coaX* nucleic acid molecule hybridizes to all or a portion of a nucleic acid molecule having the nucleotide

25 sequence set forth in SEQ ID NO:19 or hybridizes to all or a portion of a nucleic acid molecule having a nucleotide sequence that encodes a polypeptide having the amino acid sequence of any of SEQ ID NOs:7-18, 74 or 75. Such hybridization conditions are known to those skilled in the art and can be found in *Current Protocols in Molecular Biology*, Ausubel *et al.*, eds., John Wiley & Sons, Inc. (1995), sections 2, 4 and 6.

30 Additional stringent conditions can be found in *Molecular Cloning: A Laboratory Manual*, Sambrook *et al.*, Cold Spring Harbor Press, Cold Spring Harbor, NY (1989), chapters 7, 9 and 11. A preferred, non-limiting example of stringent hybridization conditions includes hybridization in 4X sodium chloride/sodium citrate (SSC), at about 65-70°C (or hybridization in 4X SSC plus 50% formamide at about 42-50°C) followed

35 by one or more washes in 1X SSC, at about 65-70°C. A preferred, non-limiting example of highly stringent hybridization conditions includes hybridization in 1X SSC, at about 65-70°C (or hybridization in 1X SSC plus 50% formamide at about 42-50°C)

followed by one or more washes in 0.3X SSC, at about 65-70°C. A preferred, non-limiting example of reduced stringency hybridization conditions includes hybridization in 4X SSC, at about 50-60°C (or alternatively hybridization in 6X SSC plus 50% formamide at about 40-45°C) followed by one or more washes in 2X SSC, at about 50-60°C. Ranges intermediate to the above-recited values, e.g., at 65-70°C or at 42-50°C are also intended to be encompassed by the present invention. SSPE (1X SSPE is 0.15 M NaCl, 10mM NaH_2PO_4, and 1.25 mM EDTA, pH 7.4) can be substituted for SSC (1X SSC is 0.15 M NaCl and 15 mM sodium citrate) in the hybridization and wash buffers; washes are performed for 15 minutes each after hybridization is complete. The hybridization temperature for hybrids anticipated to be less than 50 base pairs in length should be 5-10°C less than the melting temperature (T_m) of the hybrid, where T_m is determined according to the following equations. For hybrids less than 18 base pairs in length, $T_m(°C) = 2(\text{\# of A} + \text{T bases}) + 4(\text{\# of G} + \text{C bases})$. For hybrids between 18 and 49 base pairs in length, $T_m(°C) = 81.5 + 16.6(\log_{10}[Na^+]) + 0.41(\%G+C) - (600/N)$, where N is the number of bases in the hybrid, and $[Na^+]$ is the concentration of sodium ions in the hybridization buffer ($[Na^+]$ for 1X SSC = 0.165 M). It will also be recognized by the skilled practitioner that additional reagents may be added to hybridization and/or wash buffers to decrease non-specific hybridization of nucleic acid molecules to membranes, for example, nitrocellulose or nylon membranes, including but not limited to blocking agents (e.g., BSA or salmon or herring sperm carrier DNA), detergents (e.g., SDS), chelating agents (e.g., EDTA), Ficoll, PVP and the like. When using nylon membranes, in particular, an additional preferred, non-limiting example of stringent hybridization conditions is hybridization in 0.25-0.5M NaH_2PO_4, 7% SDS at about 65°C, followed by one or more washes at 0.02M NaH_2PO_4, 1% SDS at 65°C, see e.g., Church and Gilbert (1984) *Proc. Natl. Acad. Sci. USA* 81:1991-1995, (or, alternatively, 0.2X SSC, 1% SDS). In another preferred embodiment, an isolated nucleic acid molecule comprises a nucleotide sequence that is complementary to a *coaX* nucleotide sequence as set forth herein (e.g., is the full complement of the nucleotide sequence set forth as SEQ ID NO:19).

In another preferred embodiment, an isolated nucleic acid molecule is or includes a *coaA* gene, for example, a *Bacillus* (e.g., *B. subtilis*) *coaA* gene, or portion or fragment thereof. Exemplary isolated *coaA* nucleic acid molecules and/or genes include (1) an isolated *coaA* nucleic acid molecule or gene comprising the nucleotide sequence as set forth in any one of SEQ ID NOs:20-22; (2) an isolated *coaA* nucleic acid molecule or gene comprising a nucleotide sequence that encodes the amino acid sequence as set forth in SEQ ID NO:3; (3) an isolated *coaA* nucleic acid molecule or gene comprising a nucleotide sequence which encodes a CoaA homologue (e.g., a polypeptide having an

amino acid sequence at least about 30-35%, preferably at least about 35-40%, more preferably at least about 40-50%, and even more preferably at least about 60%, 70%, 80%, 90% or more identical to the amino acid sequence set forth as SEQ ID NO:3 and having a substantially equivalent enzymatic activity; (4) an isolated *coaA* nucleic acid

5   molecule or gene comprising a nucleotide sequence that encodes a polypeptide as set forth in any one of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:5 or SEQ ID NO:6; (5) an isolated nucleic acid molecule that hybridizes under stringent conditions to all or a portion of a nucleic acid molecule having the nucleotide sequence set forth in SEQ ID NO:20, SEQ ID NO:21 or SEQ ID NO:22 or hybridizes to all or a

10   portion of a nucleic acid molecule having a nucleotide sequence that encodes a polypeptide having the amino acid sequence of SEQ ID NO:3; and (6) an isolated nucleic acid molecule comprising a nucleotide sequence that is complementary to a *coaA* nucleotide sequence as set forth herein (*e.g.*, is the full complement of the nucleotide sequence set forth in SEQ ID NO:20, SEQ ID NO:21 or SEQ ID NO:22).

15   A nucleic acid molecule of the present invention (*e.g.*, a *coaX* nucleic acid molecule or gene or a *coaA* nucleic acid molecule or gene), can be isolated using standard molecular biology techniques and the sequence information provided herein. For example, nucleic acid molecules can be isolated using standard hybridization and cloning techniques (*e.g.*, as described in Sambrook, J., Fritsh, E. F., and Maniatis, T.

20   *Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989) or can be isolated by the polymerase chain reaction using synthetic oligonucleotide primers designed based upon the *coaX* or *coaA* nucleotide sequences set forth herein, or flanking sequences thereof. A nucleic acid of the invention (*e.g.*, a *coaX* nucleic acid molecule or

25   gene or a *coaA* nucleic acid molecule or gene), can be amplified using cDNA, mRNA or alternatively, chromosomal DNA, as a template and appropriate oligonucleotide primers according to standard PCR amplification techniques.

        Yet another embodiment of the present invention features mutant *coaX* and *coaA* nucleic acid molecules or genes. The phrase "mutant nucleic acid molecule"

30   or "mutant gene" as used herein, includes a nucleic acid molecule or gene having a nucleotide sequence which includes at least one alteration (*e.g.*, substitution, insertion, deletion) such that the polypeptide or protein that may be encoded by said mutant exhibits an activity that differs from the polypeptide or protein encoded by the wild-type nucleic acid molecule or gene. Preferably, a mutant nucleic acid molecule or mutant

35   gene (*e.g.*, a mutant *coaA* or *coaX* gene) encodes a polypeptide or protein having a reduced activity (*e.g.*, having a reduced pantothenate kinase activity) as compared to the polypeptide or protein encoded by the wild-type nucleic acid molecule or gene, for

example, when assayed under similar conditions (*e.g.*, assayed in microorganisms cultured at the same temperature). A mutant gene also can encode no polypeptide or have a reduced level of production of the wild-type polypeptide.

5   As used herein, a "reduced activity" or "reduced enzymatic activity" is one that is at least 5% less than that of the polypeptide or protein encoded by the wild-type nucleic acid molecule or gene, preferably at least 5-10% less, more preferably at least 10-25% less and even more preferably at least 25-50%, 50-75% or 75-100% less than that of the polypeptide or protein encoded by the wild-type nucleic acid molecule or gene. Ranges intermediate to the above-recited values, *e.g.*, 75-85%, 85-90%, 90-

10 95%, are also intended to be encompassed by the present invention. As used herein, a "reduced activity" or "reduced enzymatic activity" also includes an activity that has been deleted or "knocked out" (*e.g.*, approximately 100% less activity than that of the polypeptide or protein encoded by the wild-type nucleic acid molecule or gene). Activity can be determined according to any well accepted assay for measuring activity

15 of a particular protein of interest. Activity can be measured or assayed directly, for example, measuring an activity of a protein isolated or purified from a cell. Alternatively, an activity can be measured or assayed within a cell or in an extracellular medium. For example, assaying for a mutant *coaA* gene or a mutant *coaX* gene (*i.e.*, said mutant encoding a reduced pantothenate kinase activity) can be accomplished by

20 expressing the mutated gene in a microorganism, for example, a mutant microorganism which expresses pantothenate kinase in a temperature-sensitive manner, assaying the mutant gene for the ability to complement a temperature sensitive (Ts) mutant for pantothenate kinase activity. A *coaX* mutant gene or *coaA* mutant gene that encodes a "reduced pantothenate kinase activity" is one that complements the Ts mutant less

25 effectively than, for example, a corresponding wild-type *coaX* gene or *coaA* gene.

          It will be appreciated by the skilled artisan that even a single substitution in a nucleic acid or gene sequence (*e.g.*, a base substitution that encodes an amino acid change in the corresponding amino acid sequence) can dramatically affect the activity of an encoded polypeptide or protein as compared to the corresponding wild-type

30 polypeptide or protein. A mutant nucleic acid or mutant gene (*e.g.*, encoding a mutant polypeptide or protein), as defined herein, is readily distinguishable from a nucleic acid or gene encoding a protein homologue, as described above, in that a mutant nucleic acid or mutant gene encodes a protein or polypeptide having an altered activity, optionally observable as a different or distinct phenotype in a microorganism expressing said

35 mutant gene or nucleic acid or producing said mutant protein or polypeptide (*i.e.*, a mutant microorganism) as compared to a corresponding microorganism expressing the wild-type gene or nucleic acid or producing said mutant protein or polypeptide. By

contrast, a protein homologue has an identical or substantially similar activity, optionally phenotypically indiscernable when produced in a microorganism, as compared to a corresponding microorganism expressing the wild-type gene or nucleic acid. Accordingly it is not, for example, the degree of sequence identity between

5   nucleic acid molecules, genes, protein or polypeptides that serves to distinguish between homologues and mutants, rather it is the activity of the encoded protein or polypeptide that distinguishes between homologues and mutants: homologues having, for example, low (*e.g.*, 30-50% sequence identity) sequence identity yet having substantially equivalent functional activities, and mutants, for example sharing 99% sequence identity

10   yet having dramatically different or altered functional activities. Exemplary homologues are set forth in Figure 20 (*i.e.*, CoaA homologues) and in Figure 23 (*i.e.*, CoaX homologues). Exemplary mutants are described in Examples XV and XVIII herein.

15   *VIII.   Recombinant Nucleic Acid Molecules and Vectors*

The present invention further features recombinant nucleic acid molecules (*e.g.*, recombinant DNA molecules) that include nucleic acid molecules and/or genes described herein (*e.g.*, isolated nucleic acid molecules and/or genes), preferably *Bacillus* genes, more preferably *Bacillus subtilis* genes, even more preferably

20   *Bacillus subtilis* pantothenate kinase genes (*e.g.*, *coaX* genes or *coaA* genes), pantothenate biosynthetic genes (*e.g.*, genes encoding pantothenate biosynthetic enzymes, for example, *panB* genes encoding ketopantoate hydroxymethyltransferase, *panE* genes encoding ketopantoate reductase, *panC* genes encoding pantothenate synthetase, and/or *panD* genes encoding aspartate-α-decarboxylase) and/or isoleucine-

25   valine (*ilv*) biosynthetic genes (*e.g.*, *ilvBN* or *alsS* genes encoding acetohydroxyacid synthetase, *ilvC* genes encoding acetohydroxyacid isomeroreductase and/or *ilvD* genes encoding dihydroxyacid dehydratase).

The present invention further features vectors (*e.g.*, recombinant vectors) that include nucleic acid molecules (*e.g.*, isolated or recombinant nucleic acid molecules

30   and/or genes) described herein. In particular, recombinant vectors are featured that include nucleic acid sequences that encode bacterial gene products as described herein, preferably *Bacillus* gene products, more preferably *Bacillus subtilis* gene products, even more preferably *Bacillus subtilis* pantothenate biosynthetic gene products (*e.g.*, pantothenate biosynthetic enzymes, for example, ketopantoate

35   hydroxymethyltransferase, ketopantoate reductase, pantothenate synthetase, and/or aspartate-α-decarboxylase) and/or isoleucine-valine biosynthetic gene products (*e.g.*,

acetohydroxyacid synthetase, acetohydroxyacid isomeroreductase and/or dihydroxyacid dehydratase).

The term "recombinant nucleic acid molecule" includes a nucleic acid molecule (*e.g.*, a DNA molecule) that has been altered, modified or engineered such that
5   it differs in nucleotide sequence from the native or natural nucleic acid molecule from which the recombinant nucleic acid molecule was derived (*e.g.*, by addition, deletion or substitution of one or more nucleotides). Preferably, a recombinant nucleic acid molecule (*e.g.*, a recombinant DNA molecule) includes an isolated nucleic acid molecule or gene of the present invention (*e.g.*, an isolated *coaX, coaA, pan or ilv* gene)
10   operably linked to regulatory sequences.

The term "recombinant vector" includes a vector (*e.g.*, plasmid, phage, phasmid, virus, cosmid or other purified nucleic acid vector) that has been altered, modified or engineered such that it contains greater, fewer or different nucleic acid sequences than those included in the native or natural nucleic acid molecule from which
15   the recombinant vector was derived. Preferably, the recombinant vector includes a *coaX, coaA, pan or ilv* gene or recombinant nucleic acid molecule including such *coaX, coaA, pan or ilv* gene, operably linked to regulatory sequences, for example, promoter sequences, terminator sequences and/or artificial ribosome binding sites (RBSs), as defined herein.

20   The phrase "operably linked to regulatory sequence(s)" means that the nucleotide sequence of the nucleic acid molecule or gene of interest is linked to the regulatory sequence(s) in a manner which allows for expression (*e.g.*, enhanced, increased, constitutive, basal, attenuated, decreased or repressed expression) of the nucleotide sequence, preferably expression of a gene product encoded by the nucleotide
25   sequence (*e.g.*, when the recombinant nucleic acid molecule is included in a recombinant vector, as defined herein, and is introduced into a microorganism).

The term "regulatory sequence" includes nucleic acid sequences which affect (*e.g.*, modulate or regulate) expression of other nucleic acid sequences. In one embodiment, a regulatory sequence is included in a recombinant nucleic acid molecule
30   or recombinant vector in a similar or identical position and/or orientation relative to a particular gene of interest as is observed for the regulatory sequence and gene of interest as it appears in nature, *e.g.*, in a native position and/or orientation. For example, a gene of interest can be included in a recombinant nucleic acid molecule or recombinant vector operably linked to a regulatory sequence which accompanies or is adjacent to the gene
35   of interest in the natural organism (*e.g.*, operably linked to "native" regulatory sequences, for example, to the "native" promoter). Alternatively, a gene of interest can be included in a recombinant nucleic acid molecule or recombinant vector operably

linked to a regulatory sequence which accompanies or is adjacent to another (*e.g.*, a different) gene in the natural organism. Alternatively, a gene of interest can be included in a recombinant nucleic acid molecule or recombinant vector operably linked to a regulatory sequence from another organism. For example, regulatory sequences from

5   other microbes (*e.g.*, other bacterial regulatory sequences, bacteriophage regulatory sequences and the like) can be operably linked to a particular gene of interest.

In one embodiment, a regulatory sequence is a non-native or non-naturally-occurring sequence (*e.g.*, a sequence which has been modified, mutated, substituted, derivatized, deleted including sequences which are chemically synthesized).

10   Preferred regulatory sequences include promoters, enhancers, termination signals, anti-termination signals and other expression control elements (*e.g.*, sequences to which repressors or inducers bind and/or binding sites for transcriptional and/or translational regulatory proteins, for example, in the transcribed mRNA). Such regulatory sequences are described, for example, in Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular*

15   *Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989. Regulatory sequences include those which direct constitutive expression of a nucleotide sequence in a microorganism (*e.g.*, constitutive promoters and strong constitutive promoters), those which direct inducible expression of a nucleotide sequence in a microorganism (*e.g.*, inducible

20   promoters, for example, xylose inducible promoters) and those which attenuate or repress expression of a nucleotide sequence in a microorganism (*e.g.*, attenuation signals or repressor sequences). It is also within the scope of the present invention to regulate expression of a gene of interest by removing or deleting regulatory sequences. For example, sequences involved in the negative regulation of transcription can be removed

25   such that expression of a gene of interest is enhanced.

In one embodiment, a recombinant nucleic acid molecule or recombinant vector of the present invention includes a nucleic acid sequence or gene that encodes at least one bacterial gene product (*e.g.*, a pantothenate biosynthetic enzyme, an isoleucine-valine biosynthetic enzyme, or a CoaA biosynthetic enzyme, for example CoaA or

30   CoaX) operably linked to a promoter or promoter sequence. Preferred promoters of the present invention include *Bacillus* promoters and/or bacteriophage promoters (*e.g.*, bacteriophage which infect *Bacillus*). In one embodiment, a promoter is a *Bacillus* promoter, preferably a strong *Bacillus* promoter (*e.g.*, a promoter associated with a biochemical housekeeping gene in *Bacillus* or a promoter associated with a glycolytic

35   pathway gene in *Bacillus*). In another embodiment, a promoter is a bacteriophage promoter. In a preferred embodiment, the promoter is from the bacteriophage SP01. In a particularly preferred embodiment, a promoter is selected from the group consisting of

P_{15}, P_{26} or P_{veg}, for example, the promoters set forth in SEQ ID NO:39, SEQ ID NO:40 or SEQ ID NO:41. Additional preferred promoters include *tef* (the translational elongation factor (TEF) promoter) and *pyc* (the pyruvate carboxylase (PYC) promoter), which promote high level expression in *Bacillus* (*e.g.*, *Bacillus subtilis*). Additional

5 preferred promoters, for example, for use in Gram positive microorganisms include, but are not limited to, the *amyE* promoter or phage SP02 promoters. Additional preferred promoters, for example, for use in Gram negative microorganisms include, but are not limited to *tac, trp, tet, trp-tet, lpp, lac, lpp-lac, lacIq, T7, T5, T3, gal, trc, ara, SP6, λ-P_R* or *λ-P_L*.

10 In another embodiment, a recombinant nucleic acid molecule or recombinant vector of the present invention includes a terminator sequence or terminator sequences (*e.g.*, transcription terminator sequences). The term "terminator sequences" includes regulatory sequences which serve to terminate transcription of a gene. Terminator sequences (or tandem transcription terminators) can further serve to stabilize

15 mRNA (*e.g.*, by adding structure to mRNA), for example, against nucleases.

In yet another embodiment, a recombinant nucleic acid molecule or recombinant vector of the present invention includes sequences which allow for detection of the vector containing said sequences (*i.e.*, detectable and/or selectable markers), for example, sequences that overcome auxotrophic mutations, for example,

20 *ura3* or *ilvE*, fluorescent markers, and/or colorimetric markers (*e.g.*, *lacZ*/β-galactosidase), and/or antibiotic resistance genes (*e.g.*, *amp* or *tet*).

In yet another embodiment, a recombinant nucleic acid molecule or recombinant vector of the present invention includes an artificial ribosome binding site (RBS). The term "artificial ribosome binding site (RBS)" includes a site within an

25 mRNA molecule (*e.g.*, coded within DNA) to which a ribosome binds (*e.g.*, to initiate translation) which differs from a native RBS (*e.g.*, a RBS found in a naturally-occurring gene) by at least one nucleotide. Preferred artificial RBSs include about 5-6, 7-8, 9-10, 11-12, 13-14, 15-16, 17-18, 19-20, 21-22, 23-24, 25-26, 27-28, 29-30 or more nucleotides of which about 1-2, 3-4, 5-6, 7-8, 9-10, 11-12, 13-15 or more differ from the

30 native RBS (*e.g.*, the native RBS of a gene of interest). Preferably, nucleotides which differ are substituted such that they are identical to one or more nucleotides of an ideal RBS (*e.g.*, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47 or SEQ ID NO:48), when optimally aligned for comparisons. Artificial RBSs can be used to replace the naturally-occurring or native RBS associated with a particular gene.

35 Artificial RBSs preferably increase translation of a particular gene. Preferred artificial RBSs (*e.g.*, RBSs for increasing the translation of *panB*, for example, of *B. subtilis panB*) are depicted in Table IA (*e.g.*, SEQ ID NO:49 and SEQ ID NO:50).

Table 1A: Preferred panB Ribosome Binding Sites

```
          10        20
 5   _____|_____|_____
     -------AGAAAGGAGGTGA            ideal RBS (SEQ ID NO:44)
     CCCTCT-AG-AAGGAGGAGAAAACATG     RBS1 (SEQ ID NO:49)
     CCCTCT-AG--AGGAGGAGAAAACATG     RBS2 (SEQ ID NO:50)
     TAAACAT-G--AGGAGGAGAAAACATG     panB native RBS (SEQ ID NO:42)
10
```

Additional preferred artificial RBSs (*e.g.*, RBSs for increasing the translation of *panD*, for example, of *B. subtilis panD*) are depicted in Table 1B (*e.g.*, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53 and SEQ ID NO:54).

15   *Table 1B: Preferred panD Ribosome Binding Sites*

```
          10        20
     _____|_____|_____
     CTAGAAAAGGAGGAATTTAAATG         pAN423 RBS (SEQ ID NO:88)
20   TTAAGAAAGGAGGTGANNNNNATG        ideal RBS  (SEQ ID NO:45)

     TTAGAAAGGAGGATTTAAATATG         new design A  (SEQ ID NO:51)
     TTAGAAAGGAGGTTTAATTAATG         new design B  (SEQ ID NO:52)
     TTAGAAAGGAGGTGATTTAAATG         new design C1 (SEQ ID NO:53)
25   TTAGAAAGGAGGTGTTTAAAATG         new design C2 (SEQ ID NO:54)
     TTAGAAAGGAGGTGANNNNNATG         ideal RBS  (SEQ ID NO:46)
```

Additional preferred artificial RBSs (*e.g.*, RBSs for increasing the translation of *panD*, for example, of *B. subtilis panD*) are depicted in Table 1C (*e.g.*,
30   SEQ ID NO:55, SEQ ID NO:56 and SEQ ID NO:57). The predicted amino acid sequence at the C-terminus of the PanC protein is shown. The start codon for PanD translation is underlined.

Table 1C: Additional Preferred panD Ribosome Binding Sites

```
35          10        20
     _____|_____|_____
     --- --A GAA AGG AGG TGA NNN NNN N ATG   ideal RBS (SEQ ID NO:47)

     ATT CGA GAA ATG GAG AGA ATA TAA T ATG   native panD RBS (SEQ ID NO:43)
40   Ile Arg Glu Met Glu Arg Ile  *   Met    SEQ ID NO:89

     --- --A GAA AGG AGG TGA NNN NNN N ATG   ideal RBS (SEQ ID NO:47)

     ATT CGA GAA AGG AGG TGA ATA TAA T ATG   NDI (SEQ ID NO:55)
45   Ile Arg Glu Arg Arg  *       Met        SEQ ID NO:90
```

```
ATT CGA GAA AGG AGG TGA ATA ATA - ATG    NDII (SEQ ID NO:56)
Ile Arg Glu Arg Arg  *              Met    SEQ ID NO:90

ATT CGT AGA AAG GAG GTG AAT TAA T ATG    NDIII (SEQ ID NO:57)
Ile Arg Arg Lys Glu Val Asn  *      Met    SEQ ID NO:91

--- --- AGA AAG GAG GTG ANN NNN N ATG    ideal RBS (SEQ ID NO:48)
```

Accordingly, in one embodiment, a vector of the present invention includes an artificial RBS as set forth in SEQ ID NO:49 or SEQ ID NO:50. In another embodiment, a vector of the present invention includes an artificial RBS as set forth in SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53 or SEQ ID NO:54. In yet another embodiment, a vector of the present invention includes an artificial RBS as set forth in SEQ ID NO:55, SEQ ID NO:56 or SEQ ID NO:57.

In another embodiment, a recombinant vector of the present invention includes sequences that enhance replication in bacteria (e.g., replication-enhancing sequences). In one embodiment, replication-enhancing sequences are derived from E. coli. In another embodiment, replication-enhancing sequences are derived from pBR322 (e.g., sequences included within the pBR322 derived portion of any of the pAN vectors as set forth in the Figures , i.e., the Not I-Not I sequences from about 5.0 kB to 9.0 kB of the vector depicted in Figure 3A).

In yet another embodiment, a recombinant vector of the present invention includes antibiotic resistance genes. The term "antibiotic resistance genes" includes sequences which promote or confer resistance to antibiotics on the host organism (e.g., Bacillus). In one embodiment, the antibiotic resistance genes are selected from the group consisting of cat (chloramphenicol resistance) genes, tet (tetracycline resistance) genes, erm (erythromycin resistance) genes, neo (neomycin resistance) genes and spec (spectinomycin resistance) genes. Recombinant vectors of the present invention can further include homologous recombination sequences (e.g., sequences designed to allow recombination of the gene of interest into the chromosome of the host organism). For example, amyE sequences can be used as homology targets for recombination into the host chromosome.

Preferred vectors of the present invention include, but are not limited to, vectors set forth in Figures 2-15, 17, 19, 22, 25 and 26. It will further be appreciated by one of skill in the art that the design of a vector can be tailored depending on such factors as the choice of microorganism to be genetically engineered, the level of expression of gene product desired and the like.

IX.   Isolated Proteins

Another aspect of the present invention features isolated proteins (*e.g.*, isolated pantothenate biosynthetic enzymes and/or valine-isoleucine biosynthetic

5   enzymes and/or isolated CoA biosynthetic enzymes, for example isolated CoaA or CoaX). In one embodiment, proteins (*e.g.*, isolated pantothenate biosynthetic enzymes and/or valine-isoleucine biosynthetic enzymes and/or isolated CoaA biosynthetic enzymes, for example isolated CoaA or CoaX) are produced by recombinant DNA techniques and can be isolated from microorganisms of the present invention by an

10   appropriate purification scheme using standard protein purification techniques. In another embodiment, proteins are synthesized chemically using standard peptide synthesis techniques.

An "isolated" or "purified" protein (*e.g.*, an isolated or purified biosynthetic enzyme) is substantially free of cellular material or other contaminating

15   proteins from the microorganism from which the protein is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized. In one embodiment, an isolated or purified protein has less than about 30% (by dry weight) of contaminating protein or chemicals, more preferably less than about 20% of contaminating protein or chemicals, still more preferably less than about 10% of

20   contaminating protein or chemicals, and most preferably less than about 5% contaminating protein or chemicals.

In a preferred embodiment, the protein or gene product is derived from *Bacillus* (*e.g.*, is *Bacillus*-derived). The term "derived from *Bacillus*" or "*Bacillus*-derived" includes a protein or gene product which is encoded by a *Bacillus* gene.

25   Preferably, the gene product is derived from a microorganism selected from the group consisting of *Bacillus subtilis, Bacillus lentimorbus, Bacillus lentus, Bacillus firmus, Bacillus pantothenticus, Bacillus amyloliquefaciens, Bacillus cereus, Bacillus circulans, Bacillus coagulans, Bacillus licheniformis, Bacillus megaterium, Bacillus pumilus, Bacillus thuringiensis*, and other Group 1 *Bacillus* species, for example, as characterized

30   by 16S rRNA type (Priest, *supra*). In another preferred embodiment, the protein or gene product is derived from *Bacillus brevis* or *Bacillus stearothermophilus*. In another preferred embodiment, the protein or gene product is derived from a microorganism selected from the group consisting of *Bacillus licheniformis, Bacillus amyloliquefaciens, Bacillus halodurans, Bacillus subtilis*, and *Bacillus pumilus*. In a particularly preferred

35   embodiment, the protein or gene product is derived from *Bacillus subtilis* (*e.g.*, is *Bacillus subtilis*-derived). The term "derived from *Bacillus subtilis*" or "*Bacillus subtilis*-derived" includes a protein or gene product which is encoded by a *Bacillus*

subtilis gene. In yet another preferred embodiment, the protein or gene product is encoded by a *Bacillus* gene homologue (*e.g.*, a gene derived from a species distinct from *Bacillus* but having significant homology to a *Bacillus* gene of the present invention, for example, a *Bacillus pan* gene or *Bacillus ilv* gene).

5 Included within the scope of the present invention are bacterial-derived proteins or gene products and/or *Bacillus*-derived proteins or gene products (*e.g.*, B. *subtilis*-derived gene products) that are encoded by naturally-occurring bacterial and/or *Bacillus* genes (*e.g.*, B. *subtilis* genes), for example, the genes identified by the present inventors, for example, *Bacillus* or B. *subtilis coaX* genes, *coaA* genes, *pan* genes and/or

10 *ilv* genes. Further included within the scope of the present invention are bacterial-derived proteins or gene products and/or *Bacillus*-derived proteins or gene products (*e.g.*, B. *subtilis*-derived gene products) that are encoded bacterial and/or *Bacillus* genes (*e.g.*, B. *subtilis* genes) which differ from naturally-occurring bacterial and/or *Bacillus* genes (*e.g.*, B. *subtilis* genes), for example, genes which have nucleic acids that are

15 mutated, inserted or deleted, but which encode proteins substantially similar to the naturally-occurring gene products of the present invention. For example, it is well understood that one of skill in the art can mutate (*e.g.*, substitute) nucleic acids which, due to the degeneracy of the genetic code, encode for an identical amino acid as that encoded by the naturally-occurring gene. Moreover, it is well understood that one of

20 skill in the art can mutate (*e.g.*, substitute) nucleic acids which encode for conservative amino acid substitutions. It is further well understood that one of skill in the art can substitute, add or delete amino acids to a certain degree without substantially affecting the function of a gene product as compared with a naturally-occurring gene product, each instance of which is intended to be included within the scope of the present

25 invention.

In a preferred embodiment, an isolated protein of the present invention (*e.g.*, an isolated pantothenate biosynthetic enzyme and/or an isolated isoleucine-valine biosynthetic enzyme and/or an isolated CoaA biosynthetic enzymes, for example isolated CoaA or CoaX) has an amino acid sequence shown in SEQ ID NO:3, SEQ ID

30 NO:9, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38 or SEQ ID NO:87. In other embodiments, an isolated protein of the present invention is a homologue of the at least one of the proteins set forth as SEQ ID NO:3, SEQ ID NO:9, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID

35 NO:36, SEQ ID NO:38 or SEQ ID NO:87 (*e.g.*, comprises an amino acid sequence at least about 30-40% identical, preferably about 40-50% identical, more preferably about 50-60% identical, and even more preferably about 60-70%, 70-80%, 80-90%, 90-95% or

more identical to the amino acid sequence of SEQ ID NO:3, SEQ ID NO:9, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38 or SEQ ID NO:87, and has an activity that is substantially similar to that of the protein encoded by the amino acid sequence of SEQ

5   ID NO:3, SEQ ID NO:9, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38 or SEQ ID NO:87, respectively.

To determine the percent homology of two amino acid sequences or of two nucleic acids, the sequences are aligned for optimal comparison purposes (e.g., gaps

10   can be introduced in the sequence of a first amino acid or nucleic acid sequence for optimal alignment with a second amino or nucleic acid sequence). When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of

15   identical positions shared by the sequences (i.e., % identity = # of identical positions/total # of positions x 100), preferably taking into account the number of gaps and size of said gaps necessary to produce an optimal alignment.

The comparison of sequences and determination of percent homology between two sequences can be accomplished using a mathematical algorithm. A

20   preferred, non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Karlin and Altschul (1990) *Proc. Natl. Acad. Sci. USA* 87:2264-68, modified as in Karlin and Altschul (1993) *Proc. Natl. Acad. Sci. USA* 90:5873-77. Such an algorithm is incorporated into the NBLAST and XBLAST programs (version 2.0) of Altschul *et al.* (1990) *J. Mol. Biol.* 215:403-10. BLAST

25   nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to protein molecules of the invention. To obtain gapped alignments for comparison

30   purposes, Gapped BLAST can be utilized as described in Altschul *et al.* (1997) *Nucleic Acids Research* 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used. See http://www.ncbi.nlm.nih.gov. Another preferred, non-limiting example of a mathematical algorithm utilized for the comparison of sequences

35   is the algorithm of Myers and Miller (1988) *Comput Appl Biosci.* 4:11-17. Such an algorithm is incorporated into the ALIGN program available, for example, at the GENESTREAM network server, IGH Montpellier, FRANCE (http://vega.igh.cnrs.fr) or

at the ISREC server (http://www.ch.embnet.org). When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used.

5   In another preferred embodiment, the percent homology between two amino acid sequences can be determined using the GAP program in the GCG software package (available at http://www.gcg.com), using either a Blossom 62 matrix or a PAM250 matrix, and a gap weight of 12, 10, 8, 6, or 4 and a length weight of 2, 3, or 4. In yet another preferred embodiment, the percent homology between two nucleic acid sequences can be accomplished using the GAP program in the GCG software package

10   (available at http://www.gcg.com), using a gap weight of 50 and a length weight of 3.

X.   Biotransformations and Bioconversions

Another aspect of the present invention includes biotransformation processes which feature recombinant microorganisms (e.g., mutant microorganisms)

15   and/or isolated CoA, pantothenate or isoleucine-valine biosynthetic enzymes described herein. The term "biotransformation process", also referred to herein as "bioconversion processes", includes biological processes which result in the production (e.g., transformation or conversion) of any compound (e.g., intermediate or product) which is upstream of a CoA, pantothenate or isoleucine-valine biosynthetic enzyme to a

20   compound (e.g., substrate, intermediate or product) which is downstream of said CoA, pantothenate or isoleucine-valine biosynthetic enzyme.

In one embodiment, the invention features a biotransformation process for the production of a panto-compound comprising contacting a microorganism which overexpresses at least one pantothenate biosynthetic enzyme with at least one

25   appropriate substrate or precursor under conditions such that said panto-compound is produced and recovering said panto-compound. In a preferred embodiment, the invention features a biotransformation process for the production of pantoate comprising contacting a microorganism which overexpresses ketopantoate reductase (the panE gene product) with an appropriate substrate (e.g., ketopantoate) under conditions such that

30   pantoate is produced and recovering said pantoate. In another preferred embodiment, the invention features a biotransformation process for the production of pantothenate comprising contacting a microorganism which overexpresses ketopantoate reductase and pantothenate synthetase with appropriate substrates (e.g., ketopantoate and β-alanine) under conditions such that pantothenate is produced and recovering said pantothenate.

35   In yet another preferred embodiment, the invention features a biotransformation process for the production of pantothenate comprising contacting a microorganism which overexpresses ketopantoate hydroxymethyltransferase, ketopantoate reductase and

pantothenate synthetase with appropriate substrates (*e.g.*, α-ketoisovalerate and β-alanine) under conditions such that pantothenate is produced and recovering said pantothenate. Preferred recombinant microorganisms for carrying out the above-described biotransformations include pantothenate kinase mutants. Conditions under which pantoate or pantothenate are produced can include any conditions which result in the desired production of pantoate or pantothenate, respectively.

In yet another embodiment, the present invention includes a method of producing β-alanine that includes culturing a microorganism which overexpresses aspartate-α-decarboxylase under conditions such that β-alanine is produced. Preferably, the aspartate-α-decarboxylase-overexpressing microorganism has a mutation in a nucleic acid sequence encoding a pantothenate biosynthetic enzyme selected from the group consisting of ketopantoate hydroxymethyltransferase, ketopantoate reductase and pantothenate synthetase.

The invention further features a method of producing β-alanine that includes contacting a composition comprising aspartate with an isolated *Bacillus* aspartate-α-decarboxylase enzyme under conditions such that β-alanine is produced (*e.g.*, an *in vitro* synthesis method).

The microorganism(s) and/or enzymes used in the biotransformation reactions are in a form allowing them to perform their intended function (*e.g.*, producing a desired compound). The microorganisms can be whole cells, or can be only those portions of the cells necessary to obtain the desired end result. The microorganisms can be suspended (*e.g.*, in an appropriate solution such as buffered solutions or media), rinsed (*e.g.*, rinsed free of media from culturing the microorganism), acetone-dried, immobilized (*e.g.*, with polyacrylamide gel or k-carrageenan or on synthetic supports, for example, beads, matrices and the like), fixed, cross-linked or permeablized (*e.g.*, have permeablized membranes and/or walls such that compounds, for example, substrates, intermediates or products can more easily pass through said membrane or wall).

Purified or unpurified CoA biosynthetic enzyme(s) (*e.g.*, CoaA and/or CoaX), pantothenate biosynthetic enzyme(s) and/or valine-isoleucine biosynthetic enzyme(s) can also be used in biotransformation reactions. The enzyme can be in a form that allows it to perform its intended function (*e.g.*, obtaining the desired compound). For example, the enzyme can be in free form or immobilized. Purified or unpurified CoA biosynthetic enzyme(s), pantothenate biosynthetic enzyme(s) and/or valine-isoleucine biosynthetic enzyme(s) can be contacted in one or more *in vitro* reactions with appropriate substrate(s) such that the desired product is produced.

With respect to at least the above-described methodologies (*e.g.*, the production methodologies of the present invention), at least one aspect of the invention features the folowing: embodiments is which the methods do not use microorganisms of the genus *Corynebacterium* and/or microorganisms of the genus *Escherichia*; embodiments in

5   which the methods do not use microorganisms selected from the group consiting of *Escherichia coli* and *Corynebacterium glutamicum*; embodiments in which the methods do not use gram negative microorganisms; embodiments in which the microorganisms utilized do not include, express or produce nucleic acid molecules, genes or proteins (*e.g.*, biosynthetic emzymes) derived from microorganisms of the genus

10   *Corynebacterium* and/or microorganisms of the genus *Escherichia*; embodiments in which the microorganisms to not include, express or produce nucleic acid molecules, genes or proteins (*e.g.*, biosynthetic emzymes) derived from microorganisms selected from the group consisting of *Escherichia coli* and *Corynebacterium glutamicum*.

15   *XI.   Screening Assays*

Because CoA is an essential factor in bacteria, proteins (*e.g.*, enzymes) involved in the biosynthesis of CoA provide valuable tools in the search for novel anti-biotics. In particular, the CoaX protein is a valuable target for identifying bacteriocidal compounds because it bears no resemblance in primary sequence to mammalian

20   pantothenate kinase enzymes. Accordingly, the present invention also provides a method (also referred to herein as a "screening assay") for identifying modulators, *i.e.*, candidate or test compounds or agents (*e.g.*, peptides, peptidomimetics, small molecules or other drugs) which bind to CoaX, or have a stimulatory or inhibitory effect on, for example, *coaX* expression or CoaX activity.

25   In one embodiment, the invention provides assays for screening candidate or test compounds which are capable of binding to CoaX proteins or a biologically active portion thereof. In another embodiment, the invention provides assays for screening candidate or test compounds which modulate the activity of CoaX proteins or biologically active portions thereof. The test compounds of the present

30   invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the 'one-bead one-compound' library method; and synthetic library methods using affinity chromatography selection. The biological library approach is

35   limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam, K.S. (1997) *Anticancer Drug Des.* 12:145).

Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt *et al.* (1993) *Proc. Natl. Acad. Sci. U.S.A.* 90:6909; Erb *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:11422; Zuckermann *et al.* (1994). *J. Med. Chem.* 37:2678; Cho *et al.* (1993) *Science* 261:1303; Carrell *et al.*

5 (1994) *Angew. Chem. Int. Ed. Engl.* 33:2059; Carell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2061; and in Gallop *et al.* (1994) *J. Med. Chem.* 37:1233. Libraries of compounds may be presented in solution (*e.g.*, Houghten (1992) *Biotechniques* 13:412-421), or on beads (Lam (1991) *Nature* 354:82-84), chips (Fodor (1993) *Nature* 364:555-556), bacteria (Ladner USP 5,223,409), spores (Ladner USP '409), plasmids (Cull *et al.*

10 (1992) *Proc Natl Acad Sci USA* 89:1865-1869) or on phage (Scott and Smith (1990) *Science* 249:386-390); (Devlin (1990) *Science* 249:404-406); (Cwirla *et al.* (1990) *Proc. Natl. Acad. Sci.* 87:6378-6382); (Felici (1991) *J. Mol. Biol.* 222:301-310); (Ladner *supra.*).

In one embodiment, an assay is a microorganism-based assay in which a

15 recombinant microorganism which expresses a CoaX protein or biologically active portion thereof is contacted with a test compound and the ability of the test compound to modulate CoaX activity is determined. Determining the ability of the test compound to modulate CoaX activity can be accomplished by monitoring, for example, intracellular phosphopanthoate or CoA concentrations or secreted pantothenate concentrations (as

20 compounds that inhibit CoaX will result in a buildup of pantothenate in the test microorganism). CoaX substrate can be labeled with a radioisotope or enzymatic label such that modulation of CoaX activity can be determined by detecting a conversion of labeled substrate to intermediate or product. For example, CoaX substrates can be labeled with ^{32}P, ^{14}C, or ^{3}H, either directly or indirectly, and the radioisotope detected

25 by direct counting of radioemmission or by scintillation counting. Determining the ability of a compound to modulate CoaX activity can alternatively be determined by detecting the induction of a reporter gene (comprising a CoA-responsive regulatory element operatively linked to a nucleic acid encoding a detectable marker, *e.g.*, luciferase), or detecting a CoA-regulated cellular response.

30 In yet another embodiment, a screening assay of the present invention is a cell-free assay in which a CoaX protein or biologically active portion thereof is contacted with a test compound *in vitro* and the ability of the test compound to bind to or modulate the activity of the CoaX protein or biologically active portion thereof is determined. In a preferred embodiment, the assay includes contacting the CoaX protein

35 or biologically active portion thereof with known substrates to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to modulate enzymatic activity of the CoaX on its substrates.

Screening assays can be accomplished in any vessel suitable for containing the microorganisms, proteins, and/or reactants. Examples of such vessels include microtiter plates, test tubes, and micro-centrifuge tubes. In more than one embodiment of the above assay methods of the present invention, it may be desirable to

5 immobilize either CoaX protein or a recombinant microorganism expressing CoaX protein to facilitate separation of products and/or substrates, as well as to accommodate automation of the assay. For example, glutathione-S-transferase/CoaX fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione derivatized microtiter plates. Other techniques for immobilizing proteins on

10 matrices (*e.g.*, biotin-conjugation and streptavidin immobilization or antibody conjugation) can also be used in the screening assays of the invention.

In another embodiment, modulators of CoaX expression are identified in a method wherein a cell is contacted with a candidate compound and the expression of *coaX* mRNA or CoaX polypeptide in the cell is determined. The level of expression in

15 the presence of the candidate compound is compared to the level of expression in the absence of the candidate compound (or to a suitable control, for example, an appropriate buffer control or standard). The candidate compound can then be identified as a modulator of *coaX* mRNA or CoaX polypeptide expression based on this comparison.

This invention further pertains to novel agents identified by the above-

20 described screening assays. Accordingly, it is within the scope of this invention to further use an agent identified as described herein in an appropriate animal model. For example, an CoaX modulating agent identified as described herein (*e.g.*, an anti-bactericidal compound) can be used in an infectious animal model to determine the efficacy, toxicity, or side effects of treatment with such an agent.

25

This invention is further illustrated by the following examples which should not be construed as limiting. The contents of all references, patents and published patent applications cited throughout this application are incorporated herein

30 by reference.

- 59 -

EXAMPLES

General Methodology:

Strains. *Bacillus subtilis* strains of the present invention are generally derived from either of two strains. The first is variously named "168", "1A1", or "RL-
5   1". The genotype is *trpC2*. This strain was derived from the wild type "Marburg" strain by mutagenesis and has been the basis of much of the molecular biology work done on *B. subtilis*. The second strain is PY79, a prototrophic derivative of 168 that was made Trp^+ by transduction from the wild type strain W23.

Media. Standard minimal medium for *B. subtilis* is comprised of 1 x
10   Spizizen salts and 0.5% glucose. Standard solid "rich medium" is Tryptone Blood Agar Broth (Difco), and standard liquid "rich medium" is VY, a mixture of veal infusion broth and yeast extract. For testing production of pantothenate in liquid test tube cultures, an enriched form of VY, called "Special VY" or "SVY" is used. For batch fermentations, SVYG and PFMG are used. The compositions of these media are given
15   below.

VY, a rich liquid medium: 25 g Difco Veal Infusion Broth, 5 g Difco Yeast Extract, 1L water (autoclave).

TBAB, a rich solid medium: 33 g Difco Tryptone Blood Agar Broth, 1L water (autoclave).

20   *MIN, a minimal medium:* 100 ml 10 x Spizizen salts; 10 ml 50% glucose; 2 ml 10% arginine HCl*; 10 ml 0.8% tryptophan**; water to 1 liter. (*In some cases, arginine is omitted or replace by sodium glutamate at 0.04% final concentration. In general, *B. subtilis* grows faster in minimal medium when certain amino acids, such as arginine, glutamine, glutamate, or proline, are added as an auxiliary nitrogen source.)
25   (**For strains that are tryptophan auxotrophs, tryptophan is routinely added to most minimal media.)

10 x Spizizen Salts: 174 g $K_2HPO_4 \cdot 3H_2O$; 20 g $(NH_4)_2SO_4$; 60 g KH_2PO_4; 10 g $Na_3Citrate \cdot 2H_2O$; 2 g $MgSO_4 \cdot 7H_2O$; water to 993 mls; then add 3.5 ml $FeCl_3$ solution and 3.5 ml Trace Elements solution.

30   *$FeCl_3$ Solution:* 4 g $FeCl_3 \cdot 6H_2O$; 197 g $Na_3Citrate \cdot 2H_2O$; water to 1 liter (filter sterilize)

Trace Elements Solution: 0.15 g $Na_2MoO_4 \cdot 2H_2O$; 2.5 g H_3BO_3; 0.7 g $CoCl_2 \cdot 6H_2O$; 0.25 g $CuSO_4 \cdot 5H_2O$; 1.6 g $MnCl_2 \cdot 4H_2O$; 0.3 g $ZnSO_4 \cdot 7H_2O$; water to 1 liter (filter sterilize).

SVY, Special VY, a supplemented* rich medium for testing pantothenate production in test tube cultures: 25 g Difco Veal Infusion Broth; 5 g Difco yeast extract; 5 g sodium glutamate; 2.7 g ammonium sulfate; 740 ml water (autoclave); add 200 ml 1 M potassium phosphate, pH 7.0; 60 ml 50% glucose. (*For testing pantothenate production in liquid SVY test tube cultures, Na α-ketoisovalerate and/or β-alanine can be added to a concentration of 5 g/L from filter-sterilized stocks.)

PFMG, a yeast extract based medium used in fermentors: 20 g Amberex 1003™ yeast extract; 5 g sodium glutamate, 2 g ammonium sulfate; 5 g tryptophan; 10 g KH_2PO_4; 20 g $K_2HPO_4 \cdot 3H_2O$; 1 g $MgCl_2 \cdot 6H_2O$; 0.1 g $CaCl_2 \cdot 2H_2O$; 1 g sodium citrate; 0.01 g $FeSO_4 \cdot 7H_2O$; 1 ml trace elements solution; 20 g glucose; add water to 1 L. Glucose or other sugars are fed as needed. Feed solutions can contain minerals, defined or food grade nutrients.

PF, a chemically defined pantothenate free medium for testing pantothenate auxotrophy: 100 ml 10 x Spizizen Salts; 100 ml 1 x Difco Pantothenate Assay Medium; 10 ml 50% glucose; water to 1 liter.

For pantothenate auxotrophs, 1 mM Na pantothenate is added to both minimal and rich media, since there is generally not enough pantothenate in rich media to support *B. subtilis pan* mutants. Amino acids are at 100 mg per liter, when used. Selection for antibiotic resistance is done with 5 mg/L chloramphenicol, 100 mg/L spectinomycin HCl, 15 mg/L tetracycline HCl, or 1 mg/L erythromycin plus 25 mg/L lincomycin.

Pantothenate Assays: Biological assay. The indicator organism, *Lactobacillus plantarum,* requires pantothenate for growth, and responds to low concentrations (μg/L). Thus, using serial dilutions, a wide range of concentrations can be assayed. Commercially available medium (*e.g.,* Pantothenate Assay Medium (PAM), Difco), can be used. However, Difco PAM supplemented with pantothenate does not support growth to the same level as obtainable using a fresh-mixed version of Pantothenate Assay Medium (FM-PAM), made up of the individual components as specified by Difco, which is accordingly, routinely used instead of the commercial product.

Before assaying *B. subtilis* culture supernatants, the *B. subtilis* cells must be either removed or killed. *B. subtilis* culture supernatants give approximately the same pantothenate titer when the supernatants are autoclaved as when they are sterile filtered. Accordingly, routine procedures involve autoclaving samples for 5 minutes prior to the biological assay.

Pantothenate Assays : HPLC assay. Pantothenic acid production is measured by HPLC with a detector wavelength of 197 nm and a reference at 450 nm. The procedure is a modification of one recommended by Hewlett-Packard for water soluble vitamins. Samples of culture broth are diluted into an equal volume of 60%

5 acetronitrile (ACN), centrifuged and filtered. Typically a further 10-fold dilution before analysis brings the final dilution to 20-fold. Higher concentrations of product are diluted further. Compounds are separated on a C18 Phenomenex 5μ Aqua 250 x 4.6 mm column with 5% acetronitrile (ACN) in 50 mM Na phosphate buffer at pH 2.5. An ACN gradient from 5% to 95% washes the column between every sample. The area of

10 the pantothenate peak is proportional to the concentration between 5 to 1000 mg/L. Other panto-compounds are also separated and quantitated by this method.

Amino Acid Analysis: HPLC assay. Amino acids present in the fermentation medium and throughout the fermentation are measured by HPLC with a detector wavelength of 338 nm and a reference at 390 nm. The procedure is a

15 modification of one recommended by Hewlett-Packard for amino acid analysis. Samples of culture broth are prepared identically as for the panto-compound analysis. Compounds are separated on a C18 Hypersil 5μ ODS 200 x 2.1 mm column. Solvent A is 20 mM Na acetate buffer at pH 7.2. Solvent B contains 40% ACN and 40% methanol. A gradient from 100% Solvent A to 100% Solvent B separates amino acids

20 and washes the column between every sample.

Batch Fermentations. Pantothenate producing strains are grown in stirred tank fermentors, for example, in CF3000 Chemap 14 liter vessels with 10 liter working volumes. Computer control and data collection is by commercial software, for example, B. Braun Biotech MFCS software. Fermentations can be batch processes but

25 are preferably sugar-limited, fed batch processes. Some media components (*e.g.* of SVYG and PMFG) are added to the fermentor and sterilized in place. Portions of the media are sterilized separately and added to the fermentors aseptically. This procedure is well known to those familiar with the art. Additional nitrogen sources in feeds are sterilized separately and added to the carbon source after cooling.

30 The initial sugar in the medium is consumed in approximately 6 hours. Afterwards, glucose or other sugars are fed with the possible addition of minerals, and defined or food grade nutrients. Alternatively, feeds are scheduled based on a consensus profile of nutritional requirements from samples taken from earlier fermentations.

- 62 -

After inoculation, agitation is set at a relatively low speed, e.g. 200 rpm. When the dissolved oxygen (pO2) falls to 30%, computer control automatically adjusts the agitation to maintain a dissolved oxygen concentration between 25 and 30% pO2.

5

EXAMPLE I: Enhanced Production of a Panto-Compound Using Bacteria Overexpressing *panBCD* Gene Products.

This Example describes the cloning of the *B. subtilis panBCD* operon and the generation of microorganisms overexpressing the *panBCD* gene products.

10   To clone the *B. subtilis panBCD* operon, a plasmid library of *B. subtilis* GP275 (a derivative of 168) genomic DNA was transformed in *E. coli* BM4062 (*birA*^{ts}), and temperature resistant clones were selected at 42°C. By comparison of restriction maps to the genome sequence, one particular clone was deduced to contain the *B. subtilis birA* gene and the adjacent *panBCD* genes. This plasmid was named pAN201.

15   To overexpress the *panBCD* operon and produce pantothenate, the native promoter of the *panBCD* operon was replaced by either of two strong, constitutive promoters derived from the *B. subtilis* bacteriophage SP01. These two promoters are named P_{26} and P_{15}. In addition, either of two artificial ribosome binding sites (RBSs) were used to replace the native *panB* RBS. These two artificial RBSs (set forth as SEQ ID NO:49 and SEQ ID NO:50) were predicted to increase translation of *panBCD*; their sequences are shown in Table 1A. Three such engineered *panBCD* expression cassettes were built into circular plasmids capable of replicating in *E. coli*. Other features of the plasmids include a strong rho-independent transcription terminator from the *E. coli* ribosomal RNA transcription unit, called T_1T_2, a Gram-positive chloramphenicol resistance gene *(cat)*, derived from pC194, and a pair of *Not*I restriction sites at the junctions between the *E. coli* replicon and the segment intended for integration into *B. subtilis*. Three plasmids of this series, pAN004, pAN005, and pAN006 were constructed. pAN004 contains the P_{26} promoter, RBS1, and a low copy *E. coli* replicon. pAN005 contains the P_{15} promoter, which in our experience is not as strong as P_{26}, RBS1, and the low copy replicon. pAN006 contains the P_{26} promoter, RBS2, and a medium copy replicon.

The three *panBCD* expression cassettes contained in the above-mentioned three plasmids were all ligated to a DNA fragment consisting of sequences that naturally occur immediately upstream from the native *panB* gene and integrated in single copy by homologous recombination into the *panBCD* locus of *B. subtilis* strains

RL-1 and PY79, replacing the wild-type operon. This was accomplished in two steps. First a deletion-substitution that replaced about two thirds of the *panB* coding region with a Gram-positive spectinomycin resistance gene (*spec*) was integrated at *panB* to yield Spec⁻, pantothenate auxotrophs. These intermediate strains were than transformed

5   with the *panBCD* expression cassettes of pAN004, pAN005, and pAN006 after ligating them to a DNA fragment containing chromosomal sequences just upstream of *panB*. Selection of the incoming cassette was for pantothenate prototrophy. The resulting strains were named PA221, PA222 and PA223 (from RL-1), and PA235, PA232 and PA233 (from PY79), respectively. An example of a plasmid that contains the joined

10   upstream sequence that is in the integrated strain in PA221 is pAN240 (see Figure 2). The nucleotide sequence of pAN240 is set forth as SEQ ID NO:76.

Polymerase chain reaction using appropriate primers was used to verify the correct chromosomal structures of these engineered strains. When extracts of strain PA221 were examined by SDS-PAGE, two proteins were found to be overexpressed.

15   One protein had an apparent molecular weight of 29,000 and the other protein appeared to be 39,000 daltons. The 29,000 dalton bands is presumably PanB (predicted molecular weight of 29,761). The larger protein band presumably represents PanC (predicted size 31,960 daltons).

The ability of these strains to produce pantothenate in test tube cultures

20   was assesed as follows. Each strain was grown in SVY medium supplemented with 5 g/L α-ketoisovalerate (α-KIV) and 5 g/L β-alanine, to ensure that these precursors were not limiting. Culture supernatants were autoclaved and assayed using the bioassay. Relative to the parent strains, RL-1 and PY79, the engineered strains produced about 8- to 30-fold more pantothenate, attaining 1 g/L pantothenate in some cases.

25

Table 2.   *Production of pantothenate by engineered B. subtilis strains in liquid test tube cultures grown in SVY medium with 5 g/L α-KIV and 5 g/L β-alanine.*

Expt.	Strain	Promoter	RBS at *pan*B	[pantothenate] mg/L
1	RL-1	Native	Native	30
	PA221	P_{26}	RBS1	990
				790
	PA222	P_{15}	RBS1	250
				250
	PA223	P_{26}	RBS2	790
				790
2	PY79	Native	Native	40
	PA235	P_{26}	RBS1	930
				860
	PA221	P_{26}	RBS1	1100
				1030

5   The P_{26} promoter was about 3- to 4-fold more effective than the P_{15} promoter, while RBS1 and RBS2 were roughly equivalent. Plasmids such as pAN004, pAN005, pAN006 can also be recombined as circles into the *B. subtilis* wild type *panBCD* locus by Campbell-type (single crossover) integration, selecting for chloramphenicol resistance at 5 mg/L. Strains obtained in this fashion produce about

10   the same amount of pantothenate as strains PA221, PA222, and PA223, respectively. pAN004 containing the P_{26} promoter, RBS1 and a low copy *E. coli* replicon, is depicted schematically in Figure 3A. The nucleotide sequence of plasmid pAN004 is set forth as SEQ ID NO:93. pAN006 containing the P_{26} promoter, RBS2 and a medium copy *E. coli* replicon, is depicted schematically in Figure 3B. The nucleotide sequence of

15   plasmid pAN006 is set forth as SEQ ID NO:94. The nucleotide sequence of *panBCD* is set forth as SEQ ID NO:59 and the predicted amino acid sequences of PanB, PanC and PanD are set forth as SEQ ID NO:24, SEQ ID NO:26 and SEQ ID NO:28, respectively. Methods for manipulating *Bacilli* are described, for example, in Harwood, C.R. and Cutting, S.M. (editors), Molecular Biological Methods for Bacillus (1990) John Wiley &

20   Sons, Ltd., Chichester, England, the content of which is incorporated herein by reference.

EXAMPLE II: Enhanced Production of a Panto-Compound Using Bacteria Overexpressing the *panE1* Gene Product – Ketopantoate Reductase.

This Example describes the cloning of the *B. subtilis panE1* gene and the
5   generation of microorganisms overexpressing the *panE1* gene product.

Pan⁻ *B. subtilis* strains (*e.g.*, *B. subtilis* mutants blocked in the synthesis
of pantothenic acid) had previously been isolated, one of which was reported to be
affected in ketopantoate reductase activity (Baigori *et al.* (1991) *J. Bacteriol.*
173:4240-4242). However, the mutations in these strains were incorrectly mapped to
10   the *purE-tre* interval of the *B. subtilis* genetic map which does not contain the *panE* or
panBCD genes. Furthermore as shown below, a *panE* mutant does not have a Pan⁻
phenotype as the *ilvC* gene product can substitute for the *panE* gene product in *B.
subtilis* as in other bacterial strains such as *E. coli*. More recently, the *S. typhimuruim
panE* gene has been located and determined to be allelic to *apbA*, a gene required for
15   anaerobic purine biosynthesis (Frodyma *et al.* (1998) *J. Biol. Chem.* 273:5572-5576). *E.
coli* carries a highly homologous gene at the same map location. Identification of the
panE genes in *E. coli* and *S. typhimurium* was complicated by the fact that the *ilvC* gene
product, acetohydroxy acid isomeroreductase, is also capable of carrying out the
ketopantoate reductase reaction. As a result, pantothenate auxotrophy is not obtained
20   unless both *panE* and *ilvC* are mutated.

To identify the *B. subtilis panE1* gene, the *B. subtilis* genome was
searched using the protein sequence of *E. coli* or *S. typhimurium* ApbA (PanE), and two
open reading frames were identified having homology to ApbA, named *ylbQ* and *ykpB*.
These genes were renamed *panE1* and *panE2*, due to their proposed function in
25   pantothenate biosynthesis. Both *panE1* and *panE2* were cloned as PCR products
generated from RL-1 genomic DNA as a template. Both genes were disrupted by either
a spectinomycin resistance gene (*spec*) or a chloramphenicol resistance gene (*cat*). The
interrupted genes were each integrated by double crossover into PY79 to give PA240
(*ΔpanE1::spec*) and PA241 (*ΔpanE2::cat*). Neither of these strains were pantothenate
30   auxotrophs when tested on pantothenate-free (PF) plates, although PA240 containing
ΔpanE1::spec grew slightly more slowly on TBAB without added pantothenate than
with a 1 mM pantothenate supplement. By comparison, a *ΔpanB::spec* strain does not
produce single colonies on TBAB, presumably because *B. subtilis* has no active uptake
system for pantothenate.
35   It was hypothesized that the *B. subtilis* gene, *ilvC*, could function for
panE as had been shown for *E. coli*. Accordingly, the *panE1* and *panE2* disruptions

were introduced into a strain, CU550, which is reported to be *trpC2 ilvC4 leuC124*. Both the single *panE1* and the double *panE1, panE2* disruptants were pantothenate auxotrophs on PF medium.

5   *Table 3.*   *Phenotypes of various **panE1** and **panE2** mutants on rich and defined media.*

Strain	Medium	Growth*: - pan	+ pan
PY79	TBAB	+++	+++
	PF	++	++
PA240	TBAB spec	+	+++
	PF	++	++
PA241	TBAB cam	+++	+++
	PF	++	++
CU550	TBAB	+++	+++
	PF	++	++
PA256	TBAB spec	-	+++
	PF	-	++
PA258	TBAB spec, cam	-	+++
	PF	-	++

*Each "+" represents about 1 mm of colony diameter after overnight at 37°C.

Thus, mutating both *panE1* and *ilvC* results in pantothenate auxotrophy, 10   while mutating only *panE1* does not, similar to what has been reported for *E.coli* and *S. typhimurium*.

Next, the quantitative effect of *panE1* and *panE2* knockouts in a pantothenate overproducing strain (PA235 described herein) was examined. The *panE1* and *panE2* disruptions were introduced into PA235, either singly or together to produce 15   PA245 (Δ*panE1*::spec), PA248 (Δ*panE2*::cat) and PA244 (Δ*panE1*::cat, Δ*panE2*::spec). The effect of each mutation on pantothenate production was then tested in liquid test tube cultures.

Table 4. *Pantothenate production by PA235 derivatives containing* **panE1** *and* **panE2** *disruptions.*

Strain	[pan] mg/L	% of PA235
PA235	990	(100)
PA235	940	95
PA245	59	6
PA245	82	8
PA248	1060	106
PA248	1030	104
PA244	25	3
PA244	50	5

Thus, deletion analysis indicated that the *panE1* gene contributes to over
5   90% of the pantothenate production, while deletion of *panE2* did not have a significant
effect on pantothenate production. It is therefore concluded that *panE1* accounts for
most, but not necessarily all, of the ketopantoate reductase activity in *B. subtilis*. The
rest of the ketopantoate reductase activity is predicted to be supplied by *ilvC*.

Having identified *panE1* as an important gene for pantothenate
10   production, increased *panE1* expression was tested to determine whether it could
enhance pantothenate production in strains such as PA221 or PA235. The *panE1* coding
sequence was installed downstream of the P_{26} promoter and RBS2 in a vector, pOTP61,
designed to integrate and amplify at either the *bpr* locus (a non-essential protease gene)
or at the locus of the cloned insert. The resulting plasmid, pAN236 (Figure 4) was
15   transformed into PA221, selecting for resistance to tetracycline at 15 mg/L. The
nucleotide sequence of pAN236 is set forth as SEQ ID NO:77. One transformant,
named PA236 was chosen for further study.

PA236 was shown to overexpress a protein of about 31,000 daltons,
which is close to the expected molecular weight of 33,290 daltons for *panE1* protein.
20   Briefly, whole cell extracts were prepared from PY79, RL-1, PA221,
PA221/pOTP61and PA236 (2 samples). Cell extracts were separated by gel
electrophoresis and the gels were coomassie stained to visualize proteins. In cells
engineered to overexpress *panE* (PA236-1 and PA236-2), a band was visible having an
approximate molecular weight of ~31,000 daltons (as compared to molecular weight
25   markers). Moreover, PA221 and PA236 expressed increased levels of a ~29,000 dalton

band, corresponding to the *panB* gene product, and a ~39,000 dalton band, presumably corresponding the *panC* gene product. Furthermore, *E. coli* transformed with pAN006 (Figure 3B) expressed bands correlating to the *panB* and *panC* gene products and *E. coli* transfected with PAN236 expressed a ~31,000 dalton band corresponding to the *panE*

5   gene product.

Next, PA236 was compared to PA221 carrying the empty vector pOTP61 for pantothenate production in liquid test tube cultures supplemented with 5 g/L β-alanine and 5 g/L α-KIV.

10   *Table 5.   Effect of overexpression of* **panE1** *and* **panE2** *on pantothenate production by engineered strains in liquid test tube cultures.*

Strain	Additional Plasmid	Gene Overexpressed	[Pantothenate] mg/L
PA221	pOTP61	none	1,000
			940
PA236	pAN236	*panE1*	2,030
			2,050
PA238	pAN238	*panE2*	530
			680

Overexpression of *panE1* caused a two-fold increase in pantothenate production when compared to the parent strain (*e.g.*, to slightly over 2 g/L) whereas

15   overexpression of *panE2* resulted in a strain that produced about 35% less pantothenate than the parent strain. The *panE1* nucleotide sequence and predicted amino acid sequence are set forth as SEQ ID NO:29 and SEQ ID NO:30.

EXAMPLE III: Enhanced Production of a Panto-Compound by Culturing
20   **Bacteria Overexpressing *panE1* or *panBCD* in the Presence of Valine.**

The ability of valine to function as a media supplement (*e.g.*, as a substitute for α-KIV) in strains engineered to overexpress the *panBCD* operon and *panE1* was evaluated. Valine is closely related to α-KIV by transamination, is less expensive than α-KIV, and is commercially available in kilogram quantities. Valine

25   was substituted for α-KIV in the standard liquid test tube cultures in SVY medium. The concentration of valine was varied from 5 to 50 g/L. Although valine at 5 g/L was

slightly less effective than α-KIV in promoting pantothenate production, valine at 10 or 20 g/L equaled or surpassed 5 g/L α-KIV in promoting pantothenate production.

EXAMPLES IV-X Generation of Microorganisms Capable of Producing Pantothenate in a Precursor-Independent Manner

5

B. subtilis strains such as PA221 and PA235 (engineered to overexpress panBCD) and PA236 (engineered to overexpress panBCD and panE1) need to be fed α-ketoisovalerate (α-KIV) (or valine) and aspartate (or β-alanine) to achieve maximal pantothenate production, as both these precursors are limiting for pantothenate synthesis. Accordingly, manipulated microorganisms were designed to eliminate the need to feed limiting precursors of pantothenate biosynthesis in the production of pantothenate. These strains are also useful in the production of various pantothenate biosynthetic pathway intermediates.

10

15

EXAMPLE IV: Generation of Microorganisms Capable of Producing Pantothenate in an Aspartate- (or β-Alanine) Independent Manner

The panD gene was cloned into B. subtilis expression vector pOTP61 to construct pAN423 (Figure 5). The nucleotide sequence of pAN423 is set forth as SEQ ID NO:78. The NotI restriction fragment containing panD was isolated from pAN423, self ligated and used to transform PA221. Transformants resistant to Tet^{15}, Tet^{30}, and Tet^{60} were isolated and saved for further analysis.

20

Six of the pAN423 transformants plus two control transformants were grown in SVY containing 5 g/l α-KIV with and without 10 g/l aspartate and then assayed for pantothenate production (Table 6).

25

Table 6. Effect of overproducing PanD on pantothenate production with and without added aspartate.

Culture* (PA221 transformants)	Asp (10 g/L)	TetR** (μg/ml)	OD$_{550}$	[pan] (mg/L)
pOTP61-1	-	60	8.0	76
pOTP61-2	-	60	7.7	91
423#1-1	-	15	8.5	180
423#1-2	-	15	8.0	150
423#1-3	-	30	8.3	220
423#1-4	-	30	8.5	280
423#1-5	-	60	8.9	580
423#1-6	-	60	8.8	280

pOTP61-1	+	60	7.5	380
pOTP61-2	+	60	6.9	560
423#1-1	+	15	8.5	1200
423#1-2	+	15	8.6	1000
423#1-3	+	30	8.8	1200
423#1-4	+	30	9.0	1200
423#1-5	+	60	9.0	1200
423#1-6	+	60	9.0	1200

*Test tubes cultures were grown in SVY + α-KIV (5 g/L) with Asp (10 g/L) where indicated.

**TetR = Approximate Tet-resistance of transformant

The pAN423 transformants produced at least twice the amount of
5   pantothenate as the controls (*i.e.*, to a level at or near that which was obtained in earlier
experiments by the addition of β-alanine to the culture medium). The data also show
that in the absence of added aspartate, transformants containing additional copies of the
panD gene expression cassette produce more pantothenate than the control
transformants. One of the transformants, 423#1-5, produced about five times as much
10   pantothenate as the controls. These results indicated that increased levels of PanD
protein "pull" the conversion of available aspartate towards β-alanine, and that
increasing *panD* gene expression can result in enhancement of pantothenate production
both in the presence and absence of added aspartate.

Transformant 423#1-5 was re-named strain PA401 and studied further in
15   shake flask fermentations. The shake flask medium was SVY with maltose instead of
SVY with glucose. Results of shake flask experiments agreed well with test tube
experiments during the first 24 hours. In shake flask experiments without the addition
of β-alanine, PA401 produced approximately 1.5 g/l of pantothenate in 24 hours.
Addition of β-alanine to the culture medium did not further improve pantothenate titers
20   (Table 7), indicating that with this strain and these fermentation conditions, β-alanine is
not limiting pantothenate production. In fact, when no β-alanine is fed, one can observe
that PA401 is secreting β-alanine in significant amounts into the medium.

Table 7. Shake flask cultures with strain PA401 (panD) with and without β-alanine.

	Amino acids (g/l)		24 hours		
Initial β-ala Added	β-ala	Val	pH	OD$_{600}$	Pantothenate (g/l)
0	0.7	1.5	7.5	13.7	1.5
5 g/l	7.1	1.4	7.6	12.4	1.5

Each value represents the average of duplicate 250 ml baffled flasks containing 50 ml of
medium, incubated at 37°C with shaking (200 rpm).
Base Medium: SVY with 10 g/l α-KIV, 30 g/l maltose
2% Inoculum: SVY with Tet[15] grown 24 hours.

**EXAMPLE V: Engineering the *panD* gene for Further Increased Synthesis of
Aspartate Decarboxylase and Enhanced Production of Pantothenate**

This Example describes the generation of improved ribosome binding
sites (RBSs) in the *panD* gene to increase the translation of *panD* mRNA.

*Increasing the translation of the panD gene mRNA by generation of synthetic panD
RBSs*

The RBS (SEQ ID NO:88) used to express *panD* in pAN423 is a
synthetic RBS and has been used to successfully produce other proteins in *B. subtilis* at
a high level. However, it contains six mismatches when aligned to the "ideal" *B. subtilis*
RBS (SEQ ID NO:45) (*e.g.*, an RBS having a sequence which is complementary to the
16S RNA sequence within the *B.subtilis* ribosome). (See *e.g.*, Table 1B, mismatches in
bold). Two new RBSs were designed to more closely mimic the ideal RBS. These
synthetic RBSs, named new design A (NDA) and new design B (NDB) (also referred to
herein as RBS3 and RBS4), are set forth as SEQ ID NO:51 and SEQ ID NO:52 and are
aligned with the ideal RBS in Table 1B.

Oligonucleotides corresponding to the top and bottom strands of each
new RBS were synthesized, annealed, then used to replace the RBS in pAN420,
generating plasmids pAN426 and pAN427. These constructions are illustrated in Figure
6. The presence of the NDA and NDB RBS in pAN426 and pAN427 was confirmed by
DNA sequence analysis. Next, the *panD* genes from pAN426 and pAN427 were
transferred to *B. subtilis* expression vector pOTP61 as shown in Figure 7, creating

pAN428 and pAN429. The nucleotide sequence of pAN429 is set forth as SEQ ID NO:79.

     *Not*I restriction fragments lacking the *E. coli* vector sequences were isolated from pAN428 and pAN429, self-ligated, and used to transform strain PA221 to

5   resistance to Tet[15]. Four isolates resistant to Tet[60] were picked from each transformation and assayed for pantothenate and β-alanine production along with PA221 transformed with the empty vector (pOTP61) and PA221 transformed with pAN423 (strain PA401) (see Table 8).

10   *Table 8.*   *Panthothenate production by test tube cultures of PA221 transformed with pAN428 and pAN429*

Plasmid		Medium Supplements	OD$_{550}$	Pan g/l	β-Ala g/l
pOTP61		α-KIV^5	10	UND	0.04
pAN423		α-KIV^5	10	0.4	0.04
pAN428-1	*	α-KIV^5	12	0.6	0.04
pAN428-2		α-KIV^5	11	0.5	0.03
pAN428-3		α-KIV^5	11	0.3	0.03
pAN428-4		α-KIV^5	10	0.1	UND
pAN429-1		α-KIV^5	12	0.6	0.04
pAN429-2		α-KIV^5	11	0.5	0.04
pAN429-3		α-KIV^5	11	0.6	0.05
pAN429-4	#	α-KIV^5	12	0.8	0.10
pOTP61		α-KIV^5 + Asp^{10}	11	0.5	0.08
pAN423		α-KIV^5 + Asp^{10}	12	0.9	1.32
pAN428-1	*	α-KIV^5 + Asp^{10}	12	0.8	1.97
pAN428-2		α-KIV^5 + Asp^{10}	12	0.8	1.51
pAN428-3		α-KIV^5 + Asp^{10}	12	0.9	1.02
pAN428-4		α-KIV^5 + Asp^{10}	11	0.8	0.30
pAN429-1		α-KIV^5 + Asp^{10}	12	0.8	1.78
pAN429-2		α-KIV^5 + Asp^{10}	12	0.8	1.66
pAN429-3		α-KIV^5 + Asp^{10}	12	0.8	1.78
pAN429-4	#	α-KIV^5 + Asp^{10}	13	0.8	2.28

UND: Below the limits of detection. * Renamed PA402 # Renamed PA403

When grown in medium supplemented with α-KIV at 5 g/l (α-KIV^5), the pAN428-1 transformant and all four of the pAN429 transformants produced more pantothenate than did PA401, suggesting that these transformants contain higher levels of aspartate decarboxylase activity. When grown in medium supplemented with α-KIV^5

5 and Asp^{10} none of the pAN428 or pAN429 transformants produced more pantothenate than PA401. However, the pAN428-1 transformant and all four of the pAN429 transformants produced significantly more β-alanine than did PA401. It is possible that the excess β-alanine produced from added aspartate causes inhibition of pantothenate production. Alternatively, β-alanine may accumulate because pantoate is limiting in

10 these strains.

The strains that produced the highest level of β-alanine, the pAN428-1 and pAN429-4 transformants, were renamed PA402 and PA403, respectively. These two strains were grown in SVY medium supplemented with various intermediates and reassayed for pantothenate and β-alanine production. PA221 and PA401 were included

15 as controls. The results of the assays are presented in Table 9.

Table 9.   *Pantothenate production of PA402 and PA403 in test tube cultures.*

Strain	Medium Supplements	OD$_{550}$	Pan g/l	β-Ala g/l	Val g/l
PA221	α-KIV^5	7.9	UND	UND	0.9
PA401	α-KIV^5	8.7	0.3	0.04	0.9
PA402	α-KIV^5	8.5	0.5	0.04	0.9
PA403	α-KIV^5	9.4	0.7	0.07	0.9
PA221	α-KIV^5 + Asp^{10}	9.8	0.4	0.11	0.8
PA401	α-KIV^5 + Asp^{10}	9.1	0.8	1.15	0.8
PA402	α-KIV^5 + Asp^{10}	9.4	0.8	2.02	0.8
PA403	α-KIV^5 + Asp^{10}	9.7	0.7	2.40	0.8
PA221	Pantoate^5	8.9	UND	UND	0.2
PA401	Pantoate^5	8.7	0.3	0.02	0.2
PA402	Pantoate^5	10.6	0.5	0.02	0.2
PA403	Pantoate^5	10.5	0.7	0.02	0.2
PA221	Pantoate^5 + Asp^{10}	9.5	0.4	0.06	0.2
PA401	Pantoate^5 + Asp^{10}	9.2	2.2	0.62	0.2

PA402	Pantoate^5 + Asp^{10}	9.1	2.8	1.17	0.2
PA403	Pantoate^5 + Asp^{10}	10.2	2.9	1.58	0.2

UND: Below the limits of detection.

When grown in medium supplemented with either α-KIV^5 or Pantoate^5, PA402 and PA403 produced significantly more pantothenate than did PA401. As before, even though PA402 and PA403 produced significantly more β-alanine than PA401 when grown in medium supplemented with α-KIV^5 and Asp^{10}, they did not produce a proportional increase in pantothenate. However, when grown in medium supplemented with Pantoate^5 plus Asp^{10}, both PA402 and PA403 produced significantly more pantothenate than PA401, about a 30% increase.

It can be concluded from these experiments that the improved NDA and NDB *panD* ribosome binding sites, engineered into pAN428 and pAN429, respectively, lead to increased levels of aspartate decarboxylase activity.

Increasing the translation of the panD gene mRNA by generation of synthetic panD RBSs within the panBCD operon

The native *B. subtilis panD* gene ribosome binding site (RBS) (SEQ ID NO:43), which is found in the *P26panBCD* operon cassette present in PA221 (and in other engineered pantothenate production strains described herein), is shown in Table 1C aligned with the ideal ribosome binding site (SEQ ID NO:47). The alignment shows mismatches between the native *B. subtilis panD* gene RBS, which is located within the coding sequence for PanC, and the the ideal RBS. Three new RBSs (within the P26 panBCD operon cassette) were generated to increase translation of the *panD* gene mRNA and to yield increased synthesis of aspartate decarboxylase. These synthetic RBSs (termed NDI, NDII, and NDIII, also referred to herein as RBS5, RBS6 and RBS7, respectively) are set forth as SEQ ID NO:55, SEQ ID NO:56 and SEQ ID NO:57, respectively) and are included in Table 1C. It should be noted that although changes in the *panD* RBS within the *panBCD* operon also changes the C-terminal amino acid sequence of the PanC protein encoded by that operon, an alignment of known and suspected PanC protein amino acid sequences showed that the sequence of the last nine amino acids of the *B. subtilis* PanC protein could be altered without affecting any conserved amino acid residues indicating that such changes should not reduce pantothenate synthetase activity or expression. The new RBSs were synthesized and incorporated into the *P26 panBCD* operon expression cassette as follows.

First, PCR primers were designed to contain the following elements: (1) a nucleic acid sequence encoding the first five amino acids of PanD up to and including a unique *Bsi*WI restriction site that had been previously introduced into *panD* by PCR; (2) a stop codon for *panC*, (3) at least one synthetic RBS; and (4) 30-39 bp of nucleic acid

5   sequence having 100% identity with *panC* upstream of the *panD* RBS. The primers were named TP102, TP103, and TP104 and contain the NDI, NDII, and NDIII ribosome binding sites, respectively. These three primers were used in conjunction with the 5' primer TP101, which hybridizes near the start codon of *panC*, in three independent PCR reactions to generate the NDI, NDII, and NDIII PCR products. The PCR products were

10   purified, digested with *Xba*I, then cloned into plasmid vector pASK-1BA3 which had been digested with *Xba*I and *Sma*I. The resulting plasmids were named pAN431, pAN432, and pAN433. The construction of pAN431 is illustrated in Figure 8 and is representative of all three plasmid constructions. The presence of the desired synthetic *panD* gene RBS in each new plasmid was confirmed by DNA sequencing.

15   Next, the modified *panC* genes containing the new *panD* RBSs were joined with the *panD* gene utilizing the unique *Bsi*WI restriction site. This was accomplished by isolating the appropriate *Nsi*I-*Bsi*WI restriction fragments from pAN431, pAN432, and pAN433 and ligating them with a 2395 bp *Nsi*I-*Bsi*WI restriction fragment from pAN420, which supplied the *Bsi*WI-modified *panD* gene.

20   These constructions resulted in plasmids pAN441, pAN442, and pAN443, respectively. A representative construction (pAN441) is illustrated in Figure 9. The nucleotide sequence of pAN443 is set forth as SEQ ID NO:80.

The new *panD* gene RBSs were then substituted into the *P26panBCD* operon expression cassette as follows. First, a deletion-insertion mutation which

25   removes the region of *panC* containing the *panD* RBS was created. This was constructed by digesting pAN430 with a mixture of *Bsp*E1 and *Bgl*II and recovering the 4235 bp fragment which is now missing the 3' end of *panC* and the 5' end of *panD*. This fragment was ligated with an *Ava*I-*Bam*HI restriction fragment from plasmid pECC4, which contains the chloramphenicol acetyl transferase (*cat*) gene. The 5' extension

30   produced by *Ava*I digestion is compatible with that produced by *Bsp*EI while the *Bgl*II and *Bam*HI extensions are also compatible. The resulting plasmid was named pAN440, and its construction is illustrated in Figure 10.

The resulting deletion-insertion mutation was crossed into the *P26* *panBCD* operon *via* homologous recombination by transforming PA221 with linearized

35   pAN440 and selecting for resistance to chloramphenicol on Cam[5] plates containing 1 mM pantothenate. Several transformants were tested, and were all found to require 1

mM pantothenate for growth, as expected. Two of these transformants were remaned PA408A and PA408B and were assayed for pantothenate production. Neither strain synthesized measurable quantities of pantothenate, even when grown in medium containing pantoate and β-alanine at 5 g/l, indicating that the strains are deficient in

5   pantothenate synthetase activity. Next, the new *panD* RBSs were crossed into the P_{26} *panBCD* operon by transforming PA408 with linearized pAN441, pAN442, and pAN443 plasmid DNA and selecting for growth on TBAB plates without pantothenate supplementation. A transformation with linearized pAN430 (including the native panD RBS) was included as a control and was expected to give rise to transformants identical

10   to PA221 described herein. Four isolates from each transformation were assayed for pantothenate and β-alanine production in SVY medium supplemented with various intermediates (Tables 10 and 11).

Table 10.   *Pantothenate production of PA410 - PA413 in test tube cultures.*

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Strain	RBS	Medium Supplements	OD550	Pan g/l	β-Ala g/l
PA221	native	Pantoate^5	11	UND	UND
PA410-1	native	Pantoate^5	12	UND	UND
PA410-2		Pantoate^5	12	UND	UND
PA410-3		Pantoate^5	12	UND	UND
PA410-4		Pantoate^5	12	UND	UND
PA411-1	NDI	Pantoate^5	12	0.23	UND
PA411-2		Pantoate^5	12	0.20	UND
PA411-3		Pantoate^5	12	0.19	UND
PA411-4		Pantoate^5	12	UND	UND
PA412-1	NDII	Pantoate^5	12	UND	UND
PA412-2		Pantoate^5	11	UND	UND
PA412-3		Pantoate^5	13	0.18	UND
PA412-4		Pantoate^5	12	0.18	UND

PA413-1	NDIII	Pantoate^5	12	0.18	UND
PA413-2		Pantoate^5	12	0.17	UND
PA413-3		Pantoate^5	12	0.16	UND
PA413-4		Pantoate^5	12	0.17	UND

UND: Below the limits of detection.

Table 11.   *Pantothenate production of PA410 - PA413 in test tube cultures.*

Strain	RBS	Medium Supplements	OD$_{550}$	Pan g/l	β-Ala g/l
PA221	native	Pantoate^5 + Asp^{10}	11	0.3	UND
PA410-1	native	Pantoate^5 + Asp^{10}	12	0.4	UND
PA410-2		Pantoate^5 + Asp^{10}	12	0.4	UND
PA410-3		Pantoate^5 + Asp^{10}	12	0.4	UND
PA410-4		Pantoate^5 + Asp^{10}	12	0.4	UND
PA411-1	NDI	Pantoate^5 + Asp^{10}	13	1.7	0.4
PA411-2		Pantoate^5 + Asp^{10}	13	1.7	0.4
PA411-3		Pantoate^5 + Asp^{10}	13	1.8	0.3
PA411-4		Pantoate^5 + Asp^{10}	13	0.4	UND
PA412-1	NDII	Pantoate^5 + Asp^{10}	13	0.4	UND
PA412-2		Pantoate^5 + Asp^{10}	12	0.4	UND
PA412-3		Pantoate^5 + Asp^{10}	12	1.6	0.3
PA412-4		Pantoate^5 + Asp^{10}	12	1.5	0.2
PA413-1	NDIII	Pantoate^5 + Asp^{10}	13	1.6	0.3
PA413-2		Pantoate^5 + Asp^{10}	13	1.6	0.4
PA413-3		Pantoate^5 + Asp^{10}	13	1.7	0.4
PA413-4		Pantoate^5 + Asp^{10}	13	1.7	0.4

UND: Below the limits of detection.

As expected from previous experiments using PA221, none of the transformants that contained the native *panD* RBS produced measurable quantities of pantothenate when grown in medium supplemented with pantoate. However, nine of the twelve transformants expected to contain modified *panD* RBSs produced significant

quantities of pantothenate (160-230 mg/l) under these conditions, indicating that they possess elevated levels of aspartate decarboxylase activity. When grown in medium supplemented with both pantoate and aspartate, these same nine transformants produced approximately four times more pantothenate than those with the native *panD* RBS. In

5 addition, these nine transformants accumulated measurable quantities of β-alanine (230-410 mg/l). All transformants produced roughly equivalent quantities of pantothenate when grown in medium containing pantoate and β-alanine, demonstrating that each contains a functional pantothenate synthetase.

These data demonstrate that the synthetic *panD* RBSs are about four
10 times more effective than the native *panD* RBS in directing translation of the *panD* gene mRNA and evidence the utility of such synthetic RBSs in enhancing pantothenate production. Additional approaches to increasing pantothenate production can include, for example, increasing the half-life of the *panD* gene mRNA, increasing the strength of the promoter for *panD* transcription and/or increasing the stability of the PanD protein.

15

EXAMPLE VI: Construction of Strains Containing an Integrated P_{26} panE1 Cassette without an Antibiotic Resistance Gene.

20 Example II describes the identification of the *B. subtilis panE1* gene that encodes the enzyme responsible for the majority of the ketopantoate reductase activity in *B. subtilis*. PA236 (containing the pAN236 plasmid) produced about twice as much pantothenate (2 g/l) as its parent strain, PA221 (1 g/l) in 24 hour SVY test tube cultures. PA236 was presumed to contain an amplified (~3 copies) integrated pAN236 plasmid
25 based on selection for tetracycline resistance (the tetR gene product being encoded on the pAN236 plasmid in addition to the P_{26} panE1 cassette). Also useful in the methodologies of the present invention are strains that contain a single integrated unamplifiable copy of P_{26} panE1 at the *panE1* locus, for example, without an antibiotic resistance gene in the strain. Such a strain was generated as follows.

30 A plasmid named pAN251 was derived from pAN236 by inserting additional chromosomal sequences just upstream and just downstream from the P_{26} panE1 cassette. These additional sequences, which provide homology to allow integration of the P_{26} panE1 cassette at *panE1* by double crossover, were obtained by PCR from chromosomal DNA as a template. pAN251 is shown in Figure 11. The
35 nucleotide sequence of pAN251 is set forth as SEQ ID NO:81.

Next, a strain was constructed which allowed selection for the incoming P_{26} *panE1* cassette. The strain included the following three components: (1) P_{26} *panBCD*; (2) $\Delta panE1$; and (3) *ilvC⁻*, since both *panE1* and *ilvC* must be mutated to have a Pan⁻ phenotype. The starting strain was CU550 (*trpC2, ilvC4, leuC124*). The P_{26} *panBCD* cassette from PA221 chromosomal DNA was introduced in two steps to create strain PA290. Next, $\Delta panE1::spec$ was transformed into PA290, using chromosomal DNA from strain PA240, to give strain PA294 (*trpC2, ilvC4, leuC124, P_{26} panBCD, $\Delta panE1::spec$*), which is a strict pantothenate auxotroph. Finally, PA294 was transformed with plasmid pAN251, selecting for pantothenate prototrophy, to give strain PA303. This strain was expected to have the genotype *trpC2, ilvC4, leuC124, P_{26} panBCD, P_{26} panE1*. PA303 was checked for the correct chromosomal structure at the *panE1* locus by PCR using primers that flank the P_{26} insertion just upstream of *panE1*. The PCR product from PA303 was of the expected size, with a concomitant loss of the PCR product from the wild type *panE1* gene, consistent with having obtained the desired double crossover event. Furthermore, PA303 was tetracycline sensitive, which is also consistent with the desired double crossover event, as opposed to a Campbell-type single crossover of the plasmids into the chromosome. The *trp, ilv,* and *leu* auxotrophies from the parent strain were all maintained in PA303.

In 24 hour liquid SVY test tube cultures, PA303 produced almost the same level of pantothenate as positive control PA236, and about twice as much as PA221, which does not contain engineered *panE1* as indicated in Table 12.

Table 12.   *Pantothenate production by 24 hr. test tube cultures of PA303 and controls grown in SVY plus 5 g/l α-KIV and 5 g/l β-alanine.*

Strain	OD600	[pan] g/l
PA221-1	10.9	0.85
PA221-2	10.5	0.85
PA236-1	9.5	1.74
PA236-2	9.3	1.70
PA303-1	10.8	1.66
PA303-2	10.7	1.61

EXAMPLE VII: Generation of Microorganisms Capable of Producing Pantothenate in an α-KIV (or Valine) Independent Manner

α-ketoisovalerate (α-KIV) is a rate limiting intermediate for pantothenate production in certain strains deregulated for pantothenate synthesis. Addition of either α-KIV or valine at 5 g/l increases pantothenate production about 5-fold in test tube cultures with strains such as PA221. In order to alleviate the need to feed either α-KIV or valine, strains were engineered that have an increased capacity to synthesize α-KIV.

α-KIV is produced in *B. subtilis* from pyruvate by the sequential action of three enzymes encoded by four genes, *ilvB* and *ilvN*, *ilvC*, and *ilvD*. In a wild type *B. subtilis*, three of the genes (*ilvB*, *ilvN*, and *ilvC*) are the first three genes of the large *ilv-leu* operon. The fourth gene necessary for α-KIV synthesis, *ilvD*, is located by itself elsewhere on the chromosome. The *B. subtilis ilv-leu* operon is thought to be regulated only by leucine levels. Feeding of exogenous leucine reduces transcription of the *ilv-leu* operon by about 13-fold, probably by an attenuation mechanism (Grandoni *et al.* (1992) *J. Bacteriol.* 174: 3212-3219). The only known feedback regulation in the *ilv-leu* pathway is the inhibition of the *leuA* gene product by leucine.

As a first step to deregulate the synthesis of α-KIV, a copy of the *ilvBNC* region from the wild type *B. subtilis ilv-leu* operon was isolated by PCR, and installed adjacent to the P_{26} promoter and RBS2 on a vector, pOLL8, that was designed to integrate a single P_{26} expression cassette by double recombination at the *amyE* locus. The *amyE* gene encodes a nonessential α-amylase, and is a useful locus for installing expression cassettes. The resulting plasmid, pAN267, is illustrated in Figure 12. The nucleotide sequence of pAN267 is set forth as SEQ ID NO:82. pAN267 readily gave stable transformants by double crossover at the *amyE* locus of *B. subtilis* strains, as described in detail below.

Construction of pantothenate overproducing strains that are leucine prototrophs

Initially, a *B. subtilis* strain containing *ilvC4* and *ΔpanE1* was used to introduce a single copy of P_{26} *panE1* into the chromosome without using an antibiotic resistance gene. The double mutant was required to select for the incoming P_{26} *panE1* cassette because a *ΔpanE1* mutation alone does not result in pantothenate auxotrophy. A strain named CU550 was obtained containing *ilvC4* to be used as a basis for this type of strain construction. However, CU550 also contains a closely linked *leuC124* mutation, so all strains derived from CU550 required leucine. Having shown that the combination of P_{26} *panBCD* and P_{26} *panE1* was favorable for pantothenate production,

the next step was to reassemble this combination of two cassettes in a leucine prototroph.

Accordingly, the two cassettes were combined in two different strain backgrounds, RL-1 and PY79. To introduce chromosomal P_{26} panE1 into the PY79 and RL-1 strain backgrounds without using an antibiotic resistance gene, a strategy was used that did not rely on ilvC4. (The strategy took advantage of the observation that the ΔpanE1 mutation causes a pantothenate bradytrophy, manifested by relatively small colonies on TBAB (rich) plates). First, ΔpanB::cat and ΔpanE::spec were introduced into both strain backgrounds. Next, the resulting strains were transformed simultaneously with DNA from two strains, PA221 (P_{26} panBCD) and PA303 (P_{26} panE1), selecting for Pan$^+$ on TBAB plates. Colonies of two distinct sizes grew on the selective plates, with the larger size comprising about 2% of the colonies. The larger colonies were presumed to represented co-transformants that received both P_{26} panBCD and P_{26} panE1, and that the smaller colonies had received only P_{26} panBCD. Consistent with this prediction, the larger colonies had lost both Cam^r and Spec^r, while the smaller colonies had lost only the cat gene, and retained the spec gene. Furthermore, a representative derivative of PY79 named PA327, and a representative derivative of RL-1, named PA328, both produced the elevated levels of pantothenate in test tube cultures which was about 1.6 to 1.7 g/l (Table 13).

Table 13.   *Pantothenate production of PA327, PA328, and controls from 24 hr test tube cultures grown in SVY plus 5 g/l α-KIV and β-alanine.*

Strain	Background	P_{26} panE1 copy number	[pan] g/l
PA221-1	RL-1	0	0.92
PA221-2	RL-1	0	0.95
PA236-1	RL-1	amplified (~3)	1.60
PA236-2	RL-1	amplified (~3)	1.73
PA327-1	PY79	1	1.66
PA327-2	PY79	1	1.65
PA328-1	RL-1	1	1.61
PA328-2	RL-1	1	1.91

Thus, PA327 and PA328 were concluded to contain both P_{26} panBCD and P_{26} panE1, and were used for further constructions as described below. PCR analysis confirmed the presence of the two cassettes.

5   *Installation of a stable P26 ilvBNC cassette into two lineages of pantothenate overproducing strains*

Having constructed PA327 and PA328, derivatives of PY79 and RL-1 that contain *P26 panBCD* and *P26 panE1*, and that are Leu$^+$, the next step was to introduce stable copies of *P26 ilvBNC*. This was accomplished by transforming PA327
10   and PA328 with plasmid pAN267, selecting for Spec^r. Screening by PCR showed that about 85% of the obtained transformants contain *P26 ilvBNC* integrated at *amyE* by double crossover. One transformant of PA327, named PA340, and one transformant of PA328, named PA342, were chosen for further study.

In test tube cultures grown in SVY medium plus 5 g/l β-alanine but
15   without added α-KIV, both PA340 and PA342 gave the expected increase in pantothenate production over that of PA327 and PA328, to about 1.3 to 2 g/l (Table 14).

Table 14.   *Pantothenate and valine production by PA340 and PA342, both containing* P_{26} *ilvBNC in 24 hr test tube cultures grown in SVY with 5 g/l*
20   *β-alanine and with or without 5 g/l α-KIV*

Strain	Back-ground	OD600 - α-KIV	+ α-KIV	[pan] g/l - α-KIV	+ α-KIV	[val] g/l - α-KIV	+ α-KIV
PA340-1	PY79	11.8	7.1	2.02	2.10	0.38	0.90
PA340-2	PY79	10.3	7.5	1.97	2.03	0.40	0.91
PA342-1	RL-1	10.2	8.0	1.29	1.89	0.27	0.78
PA342-2	RL-1	9.6	9.2	1.34	2.04	0.21	0.79

The two new strains also gave a slight increase in valine secretion, indicating that the *ilvBNC* genes had been deregulated. However, when the same strains
25   were grown with 5 g/l α-KIV added, a further increase in pantothenate production occurred from PA342, suggesting that α-KIV was still rate limiting in this strain background. Similar results, only with more growth and hence higher pantothenate levels, were seen in shake flask cultures (Table 15).

Table 15.   *Pantothenate and valine production by PA340 and PA342, both containing* P_{26} *ilvBNC in 24 hour shake flask cultures grown in SVY with 5 g/l β-alanine and with or without 5 g/l α-KIV.*

Strain	Back-ground	OD_{600} - α-KIV	+ α-KIV	[pan] g/l - α-KIV	+ α-KIV	[val] g/l - α-KIV	+ α-KIV
PA327	PY79	21	22	0.6	3.0	0.5	1.3
PA340-1	PY79	20	20	3.5	4.1	1.0	1.9
PA340-2	PY79	22	19	3.0	2.1	0.8	1.4
PA328	RL-1	20	16	1.4	2.7	0.6	1.3
PA342-1	RL-1	17	16	3.3	3.6	0.9	1.6
PA342-2	RL-1	18	18	3.1	4.2	0.8	1.4

EXAMPLE VIII: Increasing *panD* Copy Number in Strains Engineered to Overproduce *panE1* and the *ilvBNC* Gene Products Enhances Pantothenate Production

Experiments where β-alanine was fed to cultures of engineered *B. subtilis* strains consistently showed that β-alanine was a rate limiting intermediate in pantothenate synthesis. The effect of adding additional copies of *panD* on pantothenate production in PA340 and PA342 was examined. Strains PA340 and PA342 were transformed with chromosomal DNA isolated from PA401 with selection on plates containing 15 μg/ml of tetracycline (Tet[15] plates). Transformants derived from each parent were patched onto Tet[60] plates to identify those which were likely to contain multiple copies of the expression cassette. Twelve transformants from each transformation which grew on Tet[60] were streaked for single colonies on this medium and then assayed in SVY medium test tube cultures for pantothenate production. One transformant from each group was found to produce greater than 300 mg/l pantothenate in 24 hours. These two transformants were saved and named PA404 (PA340 strain background) and PA405 (PA342 strain background). Both strains were resistant to spectinomycin, indicating that the P_{26} *ilvBNC* expression cassette was still present at *amyE*. PCR analysis of chromosomal DNA isolated from each strain confirmed that the deregulated *panE1* gene had also been retained.

Next, PA404 and PA405 were evaluated in shake flask cultures which were grown in SVY medium containing maltose as the carbon source and supplemented with various intermediates. The cultures were grown for 24 and 48 hours and then assayed for pantothenate, β-alanine, and valine production. The results of this

experiment are presented in Table 16. Analogous shake flask culture data for the parent strains (PA340 and PA342) are included in the tables for comparison.

Table 16.   *Pantothenate production by PA404 and PA405 in shake flask cultures after 24 hours*

Strain	Medium Supplements	OD$_{600}$	Pan g/l	β-Ala g/l	Val g/l
PA340	none	20	0.4	<0.1	1.0
PA404	none	22	1.8	<0.1	0.7
PA342	none	19	0.3	0.2	0.7
PA405	none	19	1.4	0.4	0.5
PA340	β-alanine^5	18	3.6	3.2	0.6
PA404	β-alanine^5	18	2.8	5.1	0.7
PA342*	β-alanine^5	17	3.3	3.3	0.5
PA405*	β-alanine^5	19	1.3	6.5	0.6

Values are the average of duplicate flasks except where indicated by *.

In the absence of any medium supplementation, PA404 and PA405 made four to five times more pantothenate in 24 hours compared to their isogenic parent strains (Table 16). The supply of β-alanine was clearly limiting in the parent strains PA340 and PA342. Addition of amplified *P26 panD* greatly increased the supply of β-alanine.

EXAMPLE IX: Deregulation of the *B. subtilis ilvD* Gene Enhances Pantothenate Production

To deregulate expression of the *ilvD gene*, standard procedures (described above) were used to integrate the constitutive *P$_{26}$* promoter and an artificial ribosome binding site, RBS2, just upstream of the *ilvD* coding region. The *ilvD* gene maps by itself, unlinked to the *ilvBNC* operon. First, a 2.4 kb region of the RL-1 chromosome that contains the *ilvD* coding region and 730 bp of upstream sequence was cloned by PCR into a low copy (about 15 per *E. coli* cell) vector called pOK12, to give plasmid pAN257, shown in Figure 13.

Taking advantage of a natural *Eco*RI site just upstream of the native *ilvD* gene promoter, and a natural *Nco*I site at the *ilvD* start codon, an artificial sequence containing P_{26} and RBS2 was inserted into pAN257 to give pAN263 (Figure 14). The nucleotide sequence of pAN263 is set forth as SEQ ID NO:83. In parallel with this

5   construction, the *cat* gene was also inserted into pAN257, between the same upstream *Eco*RI site and a *Bgl*II site in the middle of the *ilvD* coding region, to give pAN261, which is deleted for a large portion of the *ilvD* gene (Figure 15).
Using pAN261 and pAN263, the P_{26} *ilvD* cassette could then be installed in the *B. subtilis* chromosome in two steps. In the first step, pAN261 is introduced by

10   transformation, selecting for chloramphenicol resistance, and then confirming an Ilv⁻ phenotype. In the second step, pAN263 is introduced, selecting for Ilv⁺, checking for chloramphenicol sensitivity, and confirming correct local structure by PCR.

pAN261 was first transformed into strain RL-1 (highly competent) to give strain PA343 ($\Delta ilvD::cat$), and then chromosomal DNA from PA343 was used to

15   transform PA340 and PA342 to Ilv⁻ auxotrophy, yielding strains named PA348 and PA349, respectively. Chromosomal DNA is inherently more efficient than monomeric plasmid in transforming *B. subtilis*. Similarly, pAN263 DNA was transformed into PA343 (moderately competent) to give strain PA345 (P_{26} *ilvD*), and then PA345 chromosomal DNA was used to transform PA348 and PA349 to Ilv⁺ prototrophy,

20   yielding strains PA374 and PA354, respectively.

As predicted, PA374 and PA354 gave further increases in pantothenate production, to about 2.5 to 2.9 g/l, in test tube cultures grown in SVY plus 5 g/l β-alanine (Table 17).

25   Table 17. *Pantothenate and valine production by PA374 and PA354, containing* P_{26} *ilvD, and controls, in 24 hr test tube cultures grown in SVY with 5 g/l β-alanine and with or without 5 g/l α-KIV.*

Strain	Back-ground	*ilvD* status	OD$_{600}$		[pan] g/l		[val] g/l	
			α-KIV		α-KIV		α-KIV	
			-	+	-	+	-	+
PA340	PY79	w.t.	9.2	9.0	2.14	2.23	0.38	0.90
PA348	PY79	*ilvD::cat*	11.7	10.0	0.19	2.23	0.19	0.91
PA374-1	PY79	P_{26} *ilvD*	9.1	7.3	2.93	2.40	0.58	0.87
PA374-2	PY79	P_{26} *ilvD*	8.2	7.7	2.99	2.36	0.60	0.95

PA342	RL-1	w.t.	10.2	8.0	1.29	1.89	0.27	0.78
PA349	RL-1	*ilvD::cat*	8.1	7.7	0.17	1.87	0.22	0.88
PA354-1	RL-1	P_{26} *ilvD*	9.6	9.6	2.57	2.03	0.65	1.23
PA354-2	RL-1	P_{26} *ilvD*	7.5	8.2	2.48	2.24	0.64	0.97

In the absence of added β-alanine, strains PA374 and PA354 produced only about 0.2 g/l pantothenate in test tube cultures, indicating that PanD activity is significantly rate limiting.

5   To alleviate this limitation, the amplifiable P_{26} *panD* cassette from strain PA401 was installed. PA401 chromosomal DNA was transformed into PA374 and PA354, selecting for Tet^r at 15 mg/l, to yield strains PA377 and PA365, respectively. After transformants were obtained, the strains were streaked on plates containing 30 and 60 mg/l tetracycline to reamplify the copy number of the P_{26} *panD* cassette integrated at 10   the *bpr* locus. In test tube cultures grown in SVY without α-KIV or β-alanine, a substantial improvement in pantothenate titers over those of PA374 and PA354 was obtained (Tables 18 and 19).

Table 18.   *Pantothenate production by PA365, containing amplified* P_{26} *panD, and* 15   *controls, in 24 and 36 hr test tube cultures grown in SVY-glucose without*   *β-alanine or α-KIV.*

| | | OD_{600} | | [pan] g/l | |
Strain	Relevant genotype	24 hrs.	36 hrs	24 hrs.	36 hrs.
PA342-1-1	w.t. *ilvD*	11.7	8.8	b.d.	0.27
PA342-1-2	w.t. *ilvD*	12.8	8.8	b.d.	0.26
PA354-1-1	P_{26} *ilvD*	n.d.	11.0	n.d.	0.19
PA354-1-2	P_{26} *ilvD*	n.d.	8.4	n.d.	0.20
PA365-1	P_{26} *ilvD, P_{26} panD*	9.8	10.0	1.01	2.07
PA365-2	P_{26} *ilvD, P_{26} panD*	9.9	10.4	0.96	2.09

n.d. = not determined; b.d. = below detection

- 87 -

Table 19. *Pantothenate production by PA377, containing amplified P_{26} panD, and controls, in 27 hr test tube cultures grown in SVY-glucose or SVY-maltose, without α-KIV, and with or without β-alanine.*

Strain	Relevant genotype	OD_{600} - β-ala Glucose	+ β-ala Glucose	- β-ala Maltose	+ β-ala Maltose
PA374-1	P_{26} ilvD	9.4	9.8	7.0	6.4
PA374-2	P_{26} ilvD	9.2	9.6	6.6	6.3
PA377-1	P_{26} ilvD, P_{26} panD	10.0	7.6	7.2	6.1
PA377-2	P_{26} ilvD, P_{26} panD	10.5	7.8	9.4	5.4

Strain	Relevant genotype	[pan] g/l - β-ala Glucose	+ β-ala Glucose	- β-ala Maltose	+ β-ala Maltose
PA374-1	P_{26} ilvD	0.04	2.76	0.14	1.31
PA374-2	P_{26} ilvD	0.10	2.65	0.15	1.33
PA377-1	P_{26} ilvD, P_{26} panD	1.25	2.76	1.26	1.10
PA377-2	P_{26} ilvD, P_{26} panD	1.25	2.35	1.31	1.26

In SVY with glucose, an increase in pantothenate production can still be achieved by feeding 5 g/l β-alanine suggesting that increasing *panD* expression further might increase pantothenate production. In SVY with maltose, no further increase in pantothenate was obtained by feeding β-alanine suggesting that β-alanine and/or aspartate synthesis is suppressed by glucose. Strains PA377 and PA365 have been evaluated in 10 liter fermentors, where they typically produce above 20 g/l pantothenate in 48 hours without supplemental β-alanine and α-KIV or valine, described in detail below.

EXAMPLE X: 10 liter Fermentations of Pantothenate-Producing Microbes

Engineering of the P_{26} *ilvBNC* and P_{26} *ilvD* cassettes to give strains PA342 and PA354 allowed the production of 22 and 26 g/l of pantothenate, respectively, without the addition of valine or α-KIV to the fermentation medium (Table 20). At 48 hours, both strains had secreted about 0.5 g/l of valine into the medium.

Table 20.   *10-liter fermentations of five pantothenate overproducing strains.*

Strain	Medium	Feed 40% Glucose plus	OD 600 48 hr	Valine 48 hours g/l	β-ala 48 hr g/l	Pantothenate g/L		
						36 hr	48 hr	72 hr
PA 236	SVYG	50 g/l β-ala 25 g/l α-KIV	108	added	added	16	19	21
PA 342	SVYG	50 g/l β-ala	92	0.5	added	17	22	--
PA 354	SVYG	50 g/l β-ala	90	0.5	added	19	26	--
PA 365	SVYG	25g/l YE	77	0.85	0.4	18	21	27
PA 377	SVYG	25g/l YE	85	1.5	0.5	18	22	31
PA 377	PFMG	25g/l YE	96	0.8	0.4	19	25	29
PA377	PFMG	-	71	0.7	0.1	16	21	-

5   *Pantothenate synthesis in fermentors*

With the addition of the P_{26} *panD* cassette to strains PA354 and PA374 to create strains PA365 and PA377, neither β-alanine nor α-KIV needed to be added to the fermentors. Strain PA365 produced 21 g/l pantothenate in 48 hours and 27 g/l in 72 hours with no precursors added to the medium (Table 20). PA377 was somewhat better,

10   producing 18 g/l of pantothenate in 36 hours, 22 g/l in 48 hours, and 31 g/l in 72 hours). Valine was measured at 0.85 and 1.5 g/l for strains PA365 and PA377, respectively, at 48 hours in SVYG medium. Strain PA377 maintained valine between 1-1.5 g/l throughout most of the fermentation and β-alanine between 0.2 and 0.5 g/l.

Strain PA377 was further evaluated in 10-liter fermentors in yeast extract

15   based PFMG medium. Pantothenate yields in PFMG and SVYG medium were similar. In PFMG, PA377 produced 19 g/l of pantothenate in 36 hours, 25 g/l in 48 hours, and 29 g/l in 72 hours. In SVYG, PA377 produced 18 g/L pantothenate in 36 hours, 22 g/L in 48 hours and 31 g/L in 72 hours (Table 20).

EXAMPLE XI: Converting Strain PA377 to a Tryptophan Prototroph

PA377 (Trp⁻) was transformed to Trp⁺ using chromosomal DNA from PY79 to give strain PA824. After re-amplification of the $P_{26}panD$ casette, PA824 was compared to PA377 for pantothenate production in test tube cultures grown in SVY glucose with or without 5 g/L β-alanine (Table 21).

Table 21 :   Trp⁺ derivatives of PA377: Pantothenate production in 48 hour test tube cultures grown in SVY glucose, ±β-alanine

Strain	*trpC* donor	OD$_{600}$		[pan] g/L	
		- β-alanine	+ β-alanine	- β-alanine	+ β-alanine
PA377-1	RL-1	8	8	1.5	3.4
PA377-2	RL-1	8	9	1.6	3.6
PA824-1	PY79	12	10	0.7	3.7
PA824-2	PY79	11	11	1.9	4.9

The Trp+ strains grew to slightly higher densities than PA377. In the absence of exogenous β-alanine, all of the strains produced similar levels of pantothenate, while with the addition of β-alanine, the Trp+ derivatives produced somewhat more pantothenate.

Fermentor studies with PA824

PA824 was evaluated in CF3000 Chemap 14 liter vessels with 10 liter working volumes. Formulations for two of the media used in the fermentors are given in Tables 22 and 23.

Table 22 :   *Formulation for PFMG-5 medium*

BATCH

	MATERIAL	g/L (final [])
1	Amberex 1003	10
2	Na Glutamate	5
3	(NH$_4$)$_2$SO$_4$	8
4	MAZU DF 37C	2.5

Added After Sterilization and Cool Down

1	KH$_2$PO$_4$	10
2	K$_2$HPO$_4$·3H$_2$O	20
1	Glucose	20
2	MgCl$_2$·6H$_2$O	1
3	CaCl$_2$·2H$_2$O	0.1
1	Sodium Citrate	1
2	FeSO$_4$·7H$_2$O	0.01
3	SM-1000X	1.0 ml
	H$_2$O	qs to 6000 ml

5

FEED

	MATERIAL	g/L
1	Glucose	600
2	CaCl$_2$·2H$_2$O	0.6
	H$_2$O	qs to 3000 ml

Table 23 :   *Formulation for SVY-4 medium*

BATCH

	MATERIAL	g/L (final [])
1	Veal Infusion	25
2	Yeast Extract	5
3	Na Glutamate	5
4	$(NH_4)_2SO_4$	4
5	MAZU DF 37C	2.5

Added After Sterilization and Cool Down

1	KH_2PO_4	10
2	$K_2HPO_4 \cdot 3H_2O$	20
1	Glucose	20
2	$MgCl_2 \cdot 6H_2O$	1
3	$CaCl_2 \cdot 2H_2O$	0.1
1	Sodium Citrate	1
2	$FeSO_4 \cdot 7H_2O$	0.01
3	SM-1000X	1.0 ml
	H_2O	qs to 6000 ml

5

FEED

	MATERIAL	g/L
1	Glucose	600
2	$CaCl_2 \cdot 2H_2O$	0.6
	H_2O	qs to 3000 ml

         All fermentations were glucose limited fed batch processes. Immediately
10   after inoculation, agitation was set at 200 rpm. The initial batched 2% glucose was

consumed during exponential growth. Afterwards, glucose concentrations were maintained between 0.2 and 1.0 g/L by continuous feeding of a 60% glucose solution. The variable rate feed pump was computer controlled and linked to the dissolved oxygen concentration [pO$_2$] in the tank by an algorithm. When the [pO$_2$]fell to 30%, computer

5 control began to automatically adjust the agitation rate to maintain a dissolved oxygen concentration between 25 and 30% [pO$_2$]. Computer control and data recording were by Braun MFCS software.

In one study, PA284 was grown in fermentors at two temperatures (40°C

10 and 43°C) in the medium described in Table 22. Results of two experiments demonstrated that the highest pantothenate titers at early time points were produced at 43°C. The cell mass approached 150 optical density units at OD$_{600}$ and 56 hours at 43°C, and the pantothenate titers were 21 g/L, 28 g/L and 36 g/L at 36, 48 and 72 hours respectively. In the parallel fermentation at 40°C, the cell mass approached 120 optical

15 density units at OD$_{600}$ and 56 hours, and the pantothenate titers were 18 g/L, 26 g/L and 37 g/L at 36, 48 and 72 hours, respectively.

In another study, PA824 was grown in a fermentor at 43°C in the medium described in Table 23. The cell mass exceeded 160 optical density units at OD$_{600}$ and 36 hours, and the pantothenate titers were 23 g/L, 34 g/L, 37 g/L and 40 g/L

20 at 24, 36, 48 and 60 hours, respectively. In other fermentations, increasing the amount of trace elements in the glucose feed (*e.g.*, increasing the concentration of SM from 1X to 2X) resulted in even higher titers of pantothenate.

25 **EXAMPLE XII: Identification and characterization of the *B. subtilis coaA* gene product**

The annotated version of the *B. subtilis* genome sequence available on the "Subtilist" web site contains no gene labeled as *coaA*. However a homology search using the protein sequence of *E. coli* pantothenate kinase as a query sequence gave a

30 good match with *B. subtilis* gene *yqjS*, which is annotated as "unknown; similar to pantothenate kinase." This gene appears to be the penultimate gene in an operon containing five open reading frames (Figure 18). Two of the open reading frames encode proteins which are similar to D-serine dehydratase and to "ketoacyl reductase"; the other two have no known homologies. For the open reading frame corresponding to

35 *coaA*, there are three possible start codons; each having a possible ribosome-binding site

(RBS) associated with it. The three potential *coaA* ORFs were named *coaA1, coaA2,* and *coaA3,* from longest to shortest.

All three potential *coaA* open reading frames were cloned along with their respective RBSs by PCR followed by ligation into expression plasmid pAN229.

5   pAN229 is a low copy vector in *E. coli* that provides expression from the SP01 phage P_{15} promoter and can integrate by single crossover at *bpr* with tetracycline selection. A representative resulting plasmid, pAN281, is shown in Figure 19.

To determine if the cloned putative *coaA* ORFs actually encode a pantothenate kinase activity, several isolates of all three plasmids were transformed into

10   the *E. coli* strain YH1, that contains the *coaA15(Ts)* allele. Transformants were streaked to plates incubated at 30° and 43°C to test for complementation of the temperature sensitive allele. All isolates of all three *coaA* variants, except for one isolate of pAN282, complemented well at 43˚C, indicating that all three plasmid constructs encode an active pantothenate kinase. Accordingly, it can be concluded that the *B. subtilis yqjS*

15   open reading frame codes for an active pantothenate kinase.

EXAMPLE XIII: Deletion of the *coaA* gene from the *B. subtilis* genome

The *coaA* gene of *B. subtilis* (*yqjS*) was deleted from the chromosome of

20   a *B. subtilis* strain by conventional means. The majority of the *coaA* coding sequence was deleted from a plasmid clone and replaced by a chloramphenicol resistance gene (*cat*), while leaving approximately 1 kb of upstream and downstream sequence to allow homologous recombination within the chromosome, to give plasmid pAN296 (see Figure 17). pAN296 was then used to transform a *B. subtilis* strain (PY79), selecting for

25   chloramphenicol resistance. The majority of transformants result from a double crossover event that effectively substitutes the *cat* gene for the *coaA* gene. The transformed strain containing the *coaA* deletion – *cat* insertion grew normally due the presence of a second *B. subtilis* pantothenate kinase encoding gene described herein.

30

EXAMPLE XIV: Identification and characterization of a second *B. subtilis* gene encoding pantothenate kinase activity

As described in detail in the instant specification, in order to maximize pantothenate production, it is necessary to restrict the flow of pantothenate toward

35   Coenzyme A (CoA), for example, by reducing the activity of pantothenate kinase, the first enzyme in the pathway from pantothenate to CoA. After finding that deletion of

the *coaA* gene from the chromosome of *B. subtilis* is not a lethal event (see Example XIII), it was concluded that *B. subtilis* must contain a second gene that encodes an active pantothenate kinase, since pantothenate kinase is an essential enzyme activity.

5 A second pantothenate kinase-encoding gene was identified by complementing the *E. coli* strain YH1 *(coaA15(Ts))* with a *B. subtilis* gene bank and selecting for transformants that were able to grow at 43°C. Found among the transformants were two families of plasmids that had overlapping restriction maps within each family, but not between the families. As expected, the restriction map of one family was identical to that predicted from the *B. subtilis* genome sequence for the

10 homologue of the *E. coli coaA* gene (which we named *coaA* also, see above) and surrounding sequences. The other family had a restriction map that was completely non-overlapping with the first.

DNA sequencing of the ends of the cloned inserts from the second family showed that the clones came from a region of the *B. subtilis* chromosome that includes

15 the 3' end of the *fts*H gene, the 5' end of the *sul* gene, and all of the *yacB, yacC, yacD, cysK, pabB, pabA* and *pabC* genes. None of the open reading frames of these cloned inserts showed homology to any known pantothenate kinase sequences, either prokaryotic or eukaryotic.

Several deletions were created through the *B. subtilis* genomic sequences

20 in the cloned inserts. Each deletion was tested for complementation of the *E. coli* temperature sensitive pantothenate kinase. In particular, a deletion that removed all DNA between a *Stu* I site in the cloning vector and a *Swa* I site in the *yacC* gene, leaves *yacB* as the only intact open reading frame in the cloned insert (see Figure 21). This deleted plasmid still complemented the *E. coli* pantothenate kinase mutant. However,

25 another deletion that removed DNA from the *Swa* I site in *yacC* through a *Bst*1107I site in the (already truncated) *ftsH* gene, could not complement the *E. coli* pantothenate kinase mutant. From these results, it was concluded that the *yacB* open reading frame was responsible for the complementation activity. To confirm that *yacB* is a pantothenate kinase gene, the *yacB* ORF plus 112 base pairs of downstream flanking

30 sequence was amplified by PCR in two independent reactions and cloned downstream of a constitutive promote to give plasmids pAN341 and pAN342 (Figure 22). Both pAN341 and pAN342 complemented the defect in YH1 at 44°C, while a control plasmid, which has the same backbone, but expresses *panBCD* instead of *yacB* did not. This confirmed that the *yacB* open reading frame was responsible for the

35 complementation of YH1.

As such, a novel gene that encodes pantothenate kinase activity in *B. subtilis* has been discovered that is not related by homology to any previously known pantothenate kinase gene. This gene has been renamed *coaX,* as a second, alternative gene that encodes an enzyme that catalyzes the first step in the pathway from

5   pantothenate to CoaA. Deletion of *coaX* by methods described above for deleting *coaA*, in conjunction with reduction in the activity of the CoaA enzyme, provides a means to reduce pantothenate kinase activity to the desired level.

Several homologues of the *B. subtilis coaX* gene were identified by homology searching of various publically available databases using the published *yacB*

10   (*coaX*) open reading frame sequence and predicted amino acid sequence (as set forth in SEQ ID NOs:84 and 85 respectively). In two cases *(Mycobacterium tuberculosis and Streptomyces coelicolor)* the homologous *coaX* genes are adjacent to, or almost adjacent to, pantothenate biosynthetic genes, consistent with these homologs having a role in pantothenate metabolism. The CoaX proteins show no homology to the CoaA family of

15   pantothenate kinases, nor to the eukaryotic family of pantothenate kinases exemplified by PanK of *Saccharomyces cerevisiae*.

Alignment of the amino acid sequences of several bacterial CoaX homologs with the amino acid sequence predicted from translating the *B. subtilis yacB* ORF described in the published *B. subtilis* genome sequence revealed that the CoaX

20   proteins from other bacteria contained additional amino acid residues at their carboxy-terminal ends. Moreover, these extensions beyond the end of the predicted amino acid for the *B. subtilis* gene product contained two relatively well conserved segments of sequence.

Translation of nucleotide sequences just downstream from the stop codon

25   of the *B. subtilis yacB* ORF in a different reading frame revealed the existence of amino acid sequences very similar to the carboxy-terminal extensions of the other bacterial CoaX proteins. It is thus believed that an error exists in the published DNA sequence of the *B. subtilis yacB* ORF sequence that causes a frame shift leading to an artifactual downstream amino acid sequence and premature termination.

30   The PCR-generated sequences of *B. subtilis CoaX* in pAN341 and pAN342 (described above) contain enough downstream flanking sequence to encode the putative carboxy-terminal extension described above, which is consistent with the result that the clones were functional in the complementation assay. However when the 3' PCR primer was positioned to include only the shorter *yacB* ORF predicted from the

35   published sequence, but not to include the putative carboxy-terminal extension, then the

resulting plasmids, pAN329 and pAN330 (similar in structure to pAN341 and pAN342; *see* Figure 22), did not complement the defect in YH1. This result supports the notion that the published *yacB* coding sequence contains a frame-shift error, and that the carboxy-terminal end of CoaX is necessary for pantothenate kinase activity. The

5 predicted correct nucleotide sequence for *B. subtilis coaX* is set forth as SEQ ID NO:19 and the translated amino acid sequence is set forth as SEQ ID NO:9. A multiple sequence alignment of the CoaX amino acid sequences of *B. subtilis* and 11 homologues thereof is set forth in Figure 23.

10

EXAMPLE XV: Generation of mutant *coaA* genes encoding pantothenate kinase having reduced or temperature sensitive activities

This Example describes strategies for modifying the *coaA* gene (*i.e.*, by

15 introducing point mutations) to reduce the activity of pantothenate kinase after *coaX* is deleted from the genome.

Cloning and sequencing of the temperature sensitive allele of the E. coli coaA gene.

Two *E. coli* strains, each exhibiting a different mutant CoaA phenotype,

20 were obtained from the *E. coli* Genetic Stock Center. Strain DV62 contains the *coaA15(Ts)* allele, and DV79 contains the *coaA16(Fr)* mutation. DV62 is temperature sensitive at 43°C and produces a pantothenate kinase that is temperature sensitive. DV79 was obtained by reversion of DV62 to temperature resistance, and it produces a temperature stable, feedback resistant pantothenate kinase activity. Since the DNA

25 sequences of these alleles are not available in the literature, the *coaA* genes from the two mutant strains were cloned by PCR and sequenced, in addition to a *coaA* gene from a strain that is wild type at the *coaA* locus, MM294. The PCR primer at the 5' end was designed to include the start codon plus four bases upstream, and added an arbitrarily chosen ribosome binding site (RBS). The three PCR generated fragments were each

30 ligated between the *XbaI* and *BamHI* sites of pAN229 to give pAN284 (from *coaA15(Ts)*), pAN285 (from wild type *coaA*), and pAN286 (from *coaA16(Fr)*). pAN229 is a low copy *E. coli* vector that provides expression from the P_{15} promoter and that can integrate by single crossover at *bpr* in *B. subtilis* with tetracycline selection.

All three plasmids were transformed into the *E. coli* strain YH1 for

35 complementation testing. All three plasmids complemented the temperature sensitive *coaA* mutation in *E. coli* YH1. It is presumed that the *coaA15(Ts)* gene in pAN284 is

probably significantly overexpressed relative to the normal chromosomal gene, such that the overproduction compensates for the temperature sensitive defect. Complementation of a defect by overproduction is a well-documented phenomenon in *E. coli*.

5   The *coaA* coding regions from pAN284, 285, and 286 were subcloned into pGEM7 to give pAN306, 307, and 308, respectively, for DNA sequencing. As expected, the DNA sequence of the insert in pAN307 (from wild type *coaA*) matched the *coaA* sequence from the *E. coli* genome database (GenBank™). The sequence from pAN306 contains a single base change that causes a S176L substitution (*i.e.*, a Ser → Leu substitution in the amino acid sequence set forth as SEQ ID NO:2). Interestingly,
10   the DNA sequence of the pAN308 insert, derived from the feedback resistant strain, was identical to that derived from its temperature sensitive parent (represented in pAN306). This is in accord with the genetic data that indicates that the reversion of the temperature sensitive mutation occurred at a second site unlinked to the *coaA* gene.

The S176L mutation, predicted to cause the temperature sensitive defect
15   in *E. coli* pantothenate kinase, changed a serine residue that is conserved in all known or suspected bacterial *coaA* encoded pantothenate kinases, including that of *B. subtilis* (see SEQ ID NO:3 and refer to alignment). Based on this, a serine to leucine change at the homologous residue in the *B. subtilis* pantothenate kinase is predicted to result in either a temperature sensitive enzyme or one which is less active. Accordingly, to produce a
20   mutant *B. subtilis coaA* gene, this specific change was introduced into the *B. subtilis coaA* gene. The mutant version is installed in the chromosome of a *B. subtilis* strain deleted for *coaX*, for example, and the recombinant microorganism is checked for temperature sensitivity (*e.g.*, reduced growth at 43°C). The mutation is then installed into a pantothenate overproducing strain, preferably a strain deleted for the above
25   mentioned *coaX* gene by standard methods to give strains favorable for pantothenate production in *B. subtilis*, *i.e.*, a strain that has reduced pantothenate kinase activity under typical fermentation conditions.

Additional coaA point mutations resulting in reduced pantothenate kinase activity

30   Of course it is expected that many other point mutations or combinations of more than one point mutation in *B. subtilis coaA* will also lead to reduced activity. Appropriate mutations can be generated by mutagenic polymerase chain reaction and *in vitro* recombination, and identified by screening for alleles that poorly complement the *E. coli coaA15(Ts)* mutant. An example of such a mutation of this type is a tyrosine to
35   histidine substitution at amino acid 181 of B. *subtilis coaA*, generated by mutagenic

polymerase chain reaction (see SEQ ID NO:3 and first line of the alignment of Figure 24).

Isolate pAN282A was derived from the middle-sized *B. subtilis coaA* open reading frame described in Example XII. pAN282A complemented the *E. coli*

5 *coaA15(Ts)* mutant very poorly, but nonetheless at a level that was detectable above background. As was done for the *E. coli coaA* clones, the open reading frame from pAN282A was subcloned into pGEM7 to give pAN303. The DNA sequence of the insert in pAN303 showed a single base change that led to a tyrosine to histidine amino acid change at the tyrosine corresponding to Y181 of SEQ ID NO:3. This tyrosine

10 residue is conserved in all bacterial *coaA* genes/homologues present in GenBank (Figure 24). This tyrosine residue and the serine that is altered in the *E. coli* temperature sensitive pantothenate kinase described above are separated by only three amino acid residues in a region which is highly conserved in bacterial pantothenate kinases whereas the DNA sequence of a second isolate of the middle-sized open reading frame, from

15 pAN282B, was identical to the wild type sequence from the *B. subtilis* genome sequencing project. The single base change found in pAN303 probably occurred during PCR amplification of the *coaA* gene. If this variant of *coaA2* has sufficient residual biological activity in *B. subtilis*, it may be useful in the future for providing reduced pantothenate kinase activity.

20 A preferred plasmid that can serve as a basis for mutagenizing the *coaA* open reading frame is pAN294 (see *e.g.*, Figure 25 and Example XII). Briefly, mutagenic PCR is performed using pAN294 as a template and variants of *coaA* having reduced pantothenate kinase activity are screened as described above. Alternatively, mutations such as the one isolated in pAN282A can be installed into pAN294. The

25 desired mutation is then introduced into the chromosome of a *B. subtilis* strain by transformation with the appropriate pAN294 derivative and selected for chloramphenicol resistance at 5 mg/L. Among the resulting transformants will be isolates that contain the desired mutation.

In a similar fashion, mutations that reduce the activity of the CoaX

30 enzyme can be generated and identified, and such mutations used for optimizing pantothenate production by reducing CoA production as described above.

EXAMPLE XVI: Deleting the second pantothenate kinase gene, *coaX* gene from *B. subtilis*

5

With the knowledge gained above concerning the existence and nature of *coaX*, one can create a deletion of the *coaX* open reading frame from the *B. subtilis* chromosome that will remove the encoded activity, and that will not adversely affect the expression of the genes downstream from *coaX*. In such a deleted strain, the *coaA* gene will be the only gene that encodes pantothenate kinase.

10

To delete the *coaX* gene from *B. subtilis*, plasmid pAN336 (SEQ ID NO:92), which contains upstream and downstream homology for double crossover, was constructed with a kanamycin resistance gene replacing most of the *coaX* ORF (Figure 26). Strain PY79 was transformed to kanamycin resistance by pAN336, and an isolate confirmed to have resulted from a double crossover by PCR was named PA876. As predicted, deletion of *coaX* by itself is not lethal for *B. subtilis*. Furthermore,

15

chromosomal DNA from PA876 would not transform competent PA861 (PY79 ΔcoaA ::cat) to kanamycin resistance. These results indicate that it is the combination of ΔcoaA::cat and ΔcoaX :: kan that is lethal for *B. subtilis*, confirming that *B. subtilis* contains two unlinked genes that encode pantothenate kanase, *coaA* and *coaX*, and that either gene alone is capable of supplying sufficient pantothenate kinase for a normal rate

20   of growth.

EXAMPLE XVII: Construction of a plasmid designed to allow directed mutagenesis of the *B. subtilis coaA* gene

In order to easily introduce mutated *coaA* genes into the *B. subtilis*

25   chromosome, it was necessary to install an antibiotic resistance gene adjacent to the *coaA* gene. This was accomplished by joining together in the vector pGEM5 three DNA fragments: (1) a 3.4 kb DNA sequence containing 2.5 kb of genomic sequence upstream from *coaA* and the *coaA* open reading frame(s); (2) a 1.1 kb DNA sequence containing a chloramphenicol resistance gene (*cat*); and (3) a 1.4 kb DNA sequence comprising a

30   region downstream from the operon that contains *coaA*. The resulting plasmid, named pAN294, effectively replaces the open reading frame *yqjT* (the open reading frame just downstream from *coaA*) with the *cat* gene, with enough homology flanking both sides of the *cat* gene to allow double recombination into the *B. subtilis* chromosome (Figure 25). pAN294 was transformed into *B. subtilis* strain PY79, selecting for chloramphenicol

35   resistance at 5 mg/l to give strains PA836 and PA837, which are presumably identical. PA836 and 837 were checked by diagnostic PCR to show that the *cat* gene had

integrated by double crossover, as opposed to single crossover. PA836 and PA837 grow normally, leading to the conclusion that the open reading frame *yqjT* is not essential (*i.e.*, the *yqjT* open reading frame could be deleted from strains PA836 and PA837 with no significant effect on growth or pantothenate production). Thus, variant alleles (*i.e.*,

5   mutations) of the *coaA* gane can be introduced into pAN294 and the resulting plasmids can be used to introduce the variant alleles into the chromosome of, for example, a *B. subtilis* strain.

EXAMPLE XVIII: Generation of mutant *coaX* genes encoding pantothenate
10   **kinase having reduced or temperature sensitive activities**

Mutant *coaX* genes are generated by introducing point mutations into the gene and testing the resulting mutants for the ability to complement the *E. coli* YH1 strain as described in Example XII. Preferred mutations in the *coaX* gene seqeunces are those that encode a substitution of a residue conserved among CoaX sequences from a

15   variety of bacterial sources (*e.g.*, a conserved residue set forth in Figure 23). Alternatively, random mutations in the *coaX* gene sequence are generated by mutagenic PCR and *in vitro* recombination and identified by screening for alleles that poorly complement the *E. coli coaA15(Ts)* mutant.

Mutants so generated (*i.e.*, mutants having reduced *coaX* activity) can be

20   further engineered such that the endogenous *coaA* gene is deleted (as described in Example XIII). CoaX reduced-activity mutants can also be further engineered to contain reduced-activity CoaA gene products as described in Example XV.

25   **EXAMPLE XIX: Enhanced Production of Panto-Compounds Using Bacteria Having Deletions in One or More Pantothenate Biosynthetic Enzymes**

If the desired panto-compound is not pantothenate, then an appropriate deletion of one or more of the pantothenate biosynthetic genes from a pantothenate

30   overproducing strain will provide a strain that produces said desired panto-compound. In this example, the desired panto-compound is pantoate. Starting with, for example, strain PA236, PA313 or PA824 either one or both of the *panC* and *panD* genes is deleted. In another example, ketopantoate is the desired panto-compound. Starting with, for example strain PA244, PA245 or PA824 one, two or all of the *ilvC, panE1,*

35   *panC* and *panD* genes are deleted from the starting strain. If β-alanine is the desired panto-compound, then *panB* and *panC* can be deleted, preferably in a fashion that leaves

an in frame fusion of a small portion of the 5' end of *pan*B with a small portion of the 3' end of *pan*C, from the strain PA221, PA235, PA245, or PA313. In all of the above-mentioned examples, the panto-compound producing strain will be a pantothenate auxotroph. Accordingly, the growth medium requires sufficient pantothenate for

5   adequate growth. Vectors designed to overexpress *pan*D as described above are then transformed into the above strains to further enhance β-alanine production.

The above-mentioned deletions are accomplished by methods well-known to those skilled in the art, for example, by insertion of an antibiotic resistance gene and removing sufficient sequence from the target gene(s) to inactivate said target

10   gene(s). Alternatively, removal of targeted sequences is accomplished without simultaneous introduction of an antibiotic resistance gene in said target gene and then introduced by congression (co-transformation with any other appropriate selectable DNA sequence) followed by screening for the loss of function of said target gene by replica plating.

15

Table 24 : Strains (and corresponding phenotypes) for panto-compound production

Name	Pheno type	Drug resist.	panBCD locus	panE locus	ilvD locus	amyE locus	bpr locus	Parent
PA221	Trp-		P26panBCD					
PA222			P_{15}panBCD					RL-1
PA235			P26panBCD					
PA236			P_{26}panBCD	P_{26}panE1				PA221
PA327	Trp-		P26panBCD	P26panE1				PA221
PA328	Trp-		P26panBCD	P26panE1				PA235
PA340	Trp-	Spc	P26panBCD	P26panE1		P26ilvBNC		PA327
PA342	Trp-	Spc	P26panBCD	P26panE1		P26ilvBNC		PA328
PA354	Trp-	Spc	P26panBCD	P26panE1	P26ilvD	P26ilvBNC		PA342
PA365	Trp-	Spc, Tet	P26panBCD	P26panE1	P26ilvD	P26ilvBNC	P26panD423	PA354
PA374	Trp-	Spc	P26panBCD	P26panE1	P26ilvD	P26ilvBNC		PA340
PA377	Trp-	Spc, Tet	P26panBCD	P26panE1	P26ilvD	P26ilvBNC	P26panD423	PA374
PA401	Trp-		P26panBCD				P26panD423	PA221
PA402	Trp-		P26panBCD				P26panD428	PA221
PA403	Trp-		P26panBCD				P26panD429	PA221
PA404	Trp-	Spc, Tet	P26panBCD	P26panE1		P26ilvBNC	P26panD423	PA340
PA405	Trp-	Spc, Tet	P26panBCD	P26panE1		P26ilvBNC	P26panD423	PA342
PA651	Trp-	Spc	P26panBC*D	P26panE1	P26ilvD	P26ilvBNC		PA374
PA284		Spc, Tet	P26'panBCD	P26panE1	P26ilvD	P26ilvBNC	P26panD423	PA377

5

Equivalents   Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

10

What is claimed:

1. A method of producing a panto-compound comprising culturing a microorganism which overexpresses at least one *Bacillus* pantothenate biosynthetic enzyme under conditions such that the panto-compound is produced.

2. The method of claim 1, wherein the microorganism overexpresses at least one *Bacillus subtilis* pantothenate biosynthetic enzyme.

3. The method of claim 1 or 2, wherein the pantothenate biosynthetic enzyme is selected from the group consisting of ketopantoate hydroxymethyltransferase, pantothenate synthetase, aspartate-α-decarboxylase and ketopantoate reductase.

4. The method of any one of claims 1 to 3, wherein the microorganism overexpresses at least two pantothenate biosynthetic enzymes.

5. The method of any one of claims 1 to 3, wherein the microorganism overexpresses at least three pantothenate biosynthetic enzymes.

6. The method of any one of claims 1 to 5, wherein the panto-compound is selected from the group consisting of pantothenate, pantoate, ketopantoate and β-alanine.

7. A method of producing a panto-compound comprising culturing a ketopantoate reductase-overexpressing (KPAR-O) microorganism under conditions such that the panto-compound is produced.

8. The method of claim 7, wherein the panto-compound is pantothenate or pantoate.

9. The method of claim 7 or 8, wherein the ketopantoate reductase is bacterial-derived.

10. The method of claim 7 or 8, wherein the ketopantoate reductase is derived from *Bacillus*.

11.   The method of claim 7 or 8, wherein the ketopantoate reductase is derived from *Bacillus subtilis*.

12.   The method of any one of claims 7 to 11, wherein the KPAR-O microorganism further overexpresses at least one pantothenate biosynthetic enzyme in addition to overexpressing ketopantoate reductase.

13.   The method of claim 12, wherein the KPAR-O microorganism further overexpresses at least one of ketopantoate hydroxymethyltransferase, pantothenate synthetase and aspartate-α-decarboxylase.

14.   A method of producing pantothenate in a manner independent of precursor feed comprising culturing an aspartate-α-decarboxylase-overexpressing (AαD-O) microorganism having a deregulated isoleucine-valine (*ilv*) pathway under conditions such that pantothenate is produced.

15.   A method of producing at least 2 g/L pantothenate in a manner independent of aspartate or β-alanine feed comprising culturing an aspartate-α-decarboxylase-overexpressing (AαD-O) microorganism under conditions such that pantothenate is produced.

16.   A method of producing at least 2 g/L pantothenate in a manner independent of valine or α-ketoisovalerate feed comprising culturing a microorganism having a deregulated isoleucine-valine (*ilv*) biosynthetic pathway under conditions such that pantothenate is produced.

17.   A method of producing at least 30 g/L pantothenate in a manner independent of aspartate or β-alanine feed comprising culturing an aspartate-α-decarboxylase-overexpressing (AαD-O) microorganism under conditions such that pantothenate is produced.

18.   A method of producing at least 30 g/L pantothenate in a manner independent of valine or α-ketoisovalerate feed comprising culturing a microorganism having a deregulated isoleucine-valine (*ilv*) biosynthetic pathway under conditions such that pantothenate is produced.

19.   A β-alanine independent high yield production method for producing pantothenate comprising culturing a manipulated microorganism under conditions such that pantothenate is produced at a significantly high yield.

5   20.   The method of any one of claims 14 to 19, wherein the microorganism overexpresses acetohydroxyacid synthetase or is transformed with a vector comprising an *ilvBN* nucleic acid sequence or an *alsS* sequence.

21.   The method of any one of claims 14 to 19, wherein the
10   microorganism overexpresses acetohydroxyacid isomeroreductase or is transformed with a vector comprising an *ilvC* nucleic acid sequence.

22.   The method of any one of claims 14 to 19, wherein the microorganism overexpresses dihydroxyacid dehydratase or is transformed with a vector
15   comprising an *ilvD* nucleic acid sequence.

23.   The method of any one of claims 19 to 22, wherein the microorganism overexpresses aspartate-α-decarboxylase or is transformed with a vector comprising a *panD* nucleic acid sequence.
20
24.   The method of any one of claims 14 to 23, wherein the microorganism further has a deregulated pantothenate biosynthetic pathway.

25.   The method of any one of claims 14 to 24, wherein the
25   microorganism further has at least one mutant gene selected from the group consisting of a mutant *avtA* gene, a mutant *ilvE* gene, a mutant *ansB* gene and a mutant *alsD* gene.

26.   The method of claim 24, wherein the microorganism overexpresses any of ketopantoate hydroxymethyltransferase, ketopantoate reductase,
30   pantothenate synthetase and aspartate-α-decarboxylase.

27.   The method of claim 24 or 26, wherein the microorganism is transformed with a vector comprising a *panBCD* nucleic acid sequence or a vector comprising a *panE1* nucleic acid sequence.
35

28.   The method of any one of claims 14 to 16 and 19 to 27, wherein pantothenate is produced at a level selected from the group consisting of a level greater than 10g/L, a level greater than 20g/L and a level greater than 40g/L.

29.   The method of claim 20, wherein the microorganism overexpresses acetohydroxyacid synthetase derived from *Bacillus* or is transformed with a vector comprising an *ilvBN* nucleic acid sequence or an *alsS* nucleic acid sequence derived from *Bacillus*.

30.   The method of claim 21, wherein the microorganism overexpresses acetohydroxyacid isomeroreductase derived from *Bacillus* or is transformed with a vector comprising an *ilvC* nucleic acid sequence derived from *Bacillus*.

31.   The method of claim 22, wherein the microorganism overexpresses dihydroxyacid dehydratase derived from *Bacillus* or is transformed with a vector comprising av *ilvD* nucleic acid sequence derived from *Bacillus*.

32.   The method of claim 23, wherein the microorganism overexpresses aspartate-α-decarboxylase derived from *Bacillus* or is transformed with a vector comprising a *panD* nucleic acid sequence derived from *Bacillus*.

33.   The method of claim 24 or 26, wherein the microorganism overexpresses any of ketopantoate hydroxymethyltransferase, ketopantoate reductase, pantothenate synthetase and aspartate-α-decarboxylase derived from *Bacillus*.

34.   The method of claim 27, wherein the vector comprises a *panBCD* nucleic acid sequence or a *panE1* nucleic acid sequence derived from *Bacillus*.

35.   A method of producing a panto-compound comprising contacting a composition comprising at least one pantothenate biosynthesis pathway precursor or isoleucine-valine biosynthesis pathway precursor with at least one isolated *Bacillus* enzyme selected from the group consisting of ketopantoate hydroxymethyltransferase, ketopantoate reductase, pantothenate synthetase and aspartate-α-decarboxylase, under conditions such that the panto-compound is produced.

36.   A method of producing β-alanine comprising culturing an aspartate-α-decarboxylase-overexpressing (AαD-O) microorganism under conditions such that β-alanine is produced.

37.   The method of claim 36, wherein the AαD-O microorganism has a mutation in a nucleic acid sequence encoding a pantothenate biosynthetic enzyme selected from the group consisting of ketopantoate hydroxymethyltransferase, ketopantoate reductase and pantothenate synthetase.

38.   A method of producing β-alanine comprising contacting a composition comprising aspartate with an isolated *Bacillus* aspartate-α-decarboxylase enzyme under conditions such that β-alanine is produced.

39.   A method for enhancing production of a panto-compound comprising culturing a mutant microorganism having a mutant *coaX* gene under conditions such that the panto-compound production is enhanced.

40.   The method of claim 39, wherein said recombinant microorganism has a mutant *coaA* gene.

41.   A method of producing a panto-compound comprising a pantothenate kinase mutant microorganism under conditions such that the panto-compound is produced at a significantly high yield.

42.   The method of claim 41, wherein said mutant microorganism has a mutant *coaA* gene.

43.   The method of claim 41, wherein said mutant microorganism has a mutant *coaX* gene.

44.   The method of claim 41, where said mutant microorganism has a mutant *coaA* and *coaX* gene.

45.   The method of any one of claims 39 to 44, wherein said panto-compound is selected from the group consisting of ketopantoate, pantoate or pantothenate.

46.   The method of any one of claims 39 to 44, wherein said panto-compound is pantothenate.

47.   The method of any one of claims 39 to 44, wherein said panto-compound is produced at a level selected from the group consisting of a level greater than 10g/L, a level greater than 20g/L and a level greater than 40g/L.

48.   The method of any one of claims 39 to 44, wherein said recombinant microorganism further has a deregulated pantothenate biosynthetic pathway or further has a deregulated isoleucine-valine (*ilv*) biosynthetic pathway.

49.   The method of claim any one of claims 39 to 44, wherein said recombinant microorganism further overexpresses *panD* and *panE*.

50.   The method of any one of claims 39 to 44, wherein said recombinant microorganism further has at least one mutant gene selected from the group consisting of a mutant *avtA* gene, a mutant *ilvE* gene, a mutant *ansB* gene and a mutant *alsD* gene.

51.   A method for enhancing production of a panto-compound comprising culturing a microorganism that has a deregulated pantothenate biosynthetic pathway and that also has a mutation that results in reduced pantothenate kinase activity under conditions such that the panto-compound production is enhanced.

52.   A method for identifying compounds which modulate pantothenate kinase activity comprising contacting a recombinant cell expressing pantothenate kinase encoded by the *coaX* gene with a test compound and determining the ability of the test compound to modulate pantothenate kinase activity in said cell.

53.   The method of claim 52, wherein said cell further comprises a mutant *coaA* gene encoding a pantothenate kinase having reduced activity.

54.   The method of any one of claims 1 to 51, wherein the microorganism is Gram positive.

55.   The method of any one of claims 1 to 51, wherein the microorganism is Gram negative.

56.   The method of any one of claims 1 to 51, wherein the microorganism is a microorganism belonging to a genus selected from the group consisting of *Bacillus*, *Cornyebacterium*, *Lactobacillus*, *Lactococci* and *Streptomyces*.

57.   The method of any one of claims 1 to 51 and 54 to 56, wherein the microorganism is of the genus *Bacillus*.

58.   The method of any one of claims 1 to 51 and 54 to 57, wherein the microorganism is *Bacillus subtilis*.

59.   The method of any one of claims 1 to 13, 35, 39 to 51 and 54 to 58, further comprising recovering the panto-compound.

60.   The method of any one of claims 14 to 34 and 54 to 58, further comprising recovering the pantothenate.

61.   The method of any one of claims 1 to 14, 35 , 39 to 46, 48 to 51 and 54 to 59, wherein the panto-compound is produced at a level greater than 2 g/L.

62.   A recombinant microorganism which overexpresses at least one *Bacillus* pantothenate biosynthetic enzyme.

63.   The recombinant microorganism of claim 62, which overexpresses at least one *Bacillus subtilis* pantothenate biosynthetic enzyme.

64.   The recombinant microorganism of claim 62 or 63, wherein the pantothenate biosynthetic enzyme is selected from the group consisting of ketopantoate hydroxymethyltransferase, pantothenate synthetase, aspartate-α-decarboxylase and ketopantoate reductase.

65.   The recombinant microorganism of any one of claims 62 to 64, wherein the pantothenate biosynthetic enzyme is ketopantoate reductase.

66.   A recombinant microorganism which overexpresses aspartate-α-decarboxylase and has a deregulated isoleucine-valine (*ilv*) biosynthetic pathway.

67.   A recombinant microorganism having a mutant *coaX* gene, said mutant *coaX* gene encoding reduced pantothenate kinase activity in said microorganism.

68.   The recombinant microorganism of claim 67 further having a mutant *coaA* gene, said mutant *coaA* gene encoding reduced pantothenate kinase activity in said microorganism.

69.   A recombinant microorganism having a mutant *coaX* gene and optionally having a mutant *coaA* gene, said mutant microorganism having reduced pantothenate kinase activity as compared to a microorganism having wild-type *coaA* and *coaX* genes.

70.   A recombinant microorganism comprising a vector comprising an isolated *coaX* gene.

71.   A recombinant microorganism that overproduces a panto-compound, the microorganism having a deregulated pantothenate biosynthetic pathway and having at least one mutation that results in a decrease in the capacity of the microorganism to synthesize Coenzyme A (CoA).

72.   The recombinant microorganism of claim 71, having at least one mutation that results in a reduced level of pantothenate kinase activity.

73.   The recombinant microorganism of claim 72, having a mutation in a *coaA* gene, or homologue thereof, that results in a reduced level of CoaA enzyme activity.

74.   The recombinant microorganism of claim 72, having a mutation in a *coaX* gene, or homologue thereof, that results in a reduced level of CoaX enzyme activity.

75.   The recombinant microorganism of claim 72, having a mutation in a *coaA* gene, or homologue thereof, and having a mutation in a *coaX* gene, or homologue thereof, the mutations resulting in reduced levels of CoaA enzyme activity and reduced CoaX enzyme activity.

76.   The recombinant microorganism of any one of claims 66 to 70 which further has a deregulated pantothenate biosynthetic pathway.

77.   The recombinant microorganism of any one of claims 62 to 65 and 67 to 75, further having a deregulated isoleucine-valine (*ilv*) biosynthetic pathway.

78.   The recombinant microorganism of any one of claims 62 to 77, which is Gram positive.

79.   The recombinant microorganism of claim 78 belonging to a genus selected from the group consisting of *Bacillus*, *Cornyebacterium*, *Lactobacillus*, *Lactococci* and *Streptomyces*.

80.   The recombinant microorganism of claim 79 belonging to the genus *Bacillus*.

81.   The recombinant microorganism of claim 80 which is *Bacillus subtilis*.

82.   A recombinant microorganism selected from the group consisting of PA221, PA235, PA236, PA313, PA410, PA402, PA403, PA411, PA412, PA413, PA303, PA327, PA328, PA401, PA340, PA342, PA404, PA405, PA374, PA354, PA365, PA377, PA651 and PA824.

83.   A recombinant vector for use in the production of panto-compounds comprising a nucleic acid sequence which encodes at least one *Bacillus* pantothenate biosynthetic enzyme operably linked to regulatory sequences.

84.   The vector of claim 83, comprising a nucleic acid sequence which encodes at least one *Bacillus subtilis* pantothenate biosynthetic enzyme.

85.   The vector of claim 84, wherein the nucleic acid sequence encodes at least one of ketopantoate hydroxymethyltransferase, pantothenate synthetase, aspartate-α-decarboxylase and ketopantoate reductase.

86.   A recombinant vector comprising at least one nucleic acid sequence selected from the group consisting of SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29 and SEQ ID NO:59.

87.   The vector of claim 84, wherein the nucleic acid sequence encodes ketopantoate reductase.

88.   A vector comprising a mutant *coaX* gene, said mutant encoding a pantothenate kinase enzyme having reduced activity.

89.   A vector comprising an isolated *coaX* gene.

90.   A vector comprising an isolated *Bacillus coaX* gene.

91.   A vector comprising an isolated *Bacillus subtilis coaX* gene.

92.   The vector of any one of claims 86 and 89 to 91, which further comprises regulatory sequences.

93.   The vector of any one of claims 83 to 85, 87 and 92, wherein the regulatory sequences comprise a constitutively active promoter.

94.   The vector of claim 93, wherein the constitutively active promoter comprises P_{veg} (SEQ ID NO:41), P_{15} (SEQ ID NO:39) or P_{26} (SEQ ID NO:40) sequences.

95.   The vector of claim 83, wherein the regulatory sequences comprise at least one artificial ribosome binding site (RBS).

96.   The vector of claim 95, wherein the artificial RBS comprises a nucleotide sequence selected from the group consisting of SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56 and SEQ ID NO:57.

97.   A vector selected from the group consisting of pAN004, pAN005, pAN006, pAN236, pAN423, pAN428, pAN429, pAN441, pAN442, pAN443, pAN251,

pAN267, pAN256, pAN257, pAN263, pAN240, pAN294, pAN296, pAN336, pAN341 and pAN342.

98.   A recombinant microorganism comprising the vector of claim 86 or 93.

99.   An isolated nucleic acid molecule which encodes at least one *Bacillus* pantothenate biosynthetic gene.

100.   The isolated nucleic acid molecule of claim 99 which encodes at least one *Bacillus subtilis* pantothenate biosynthetic gene.

101.   The isolated nucleic acid molecule of claim 99 or 100 which encodes ketopantoate reductase.

102.   An isolated *Bacillus* pantothenate biosynthetic enzyme polypeptide.

103.   An isolated *Bacillus subtilis* pantothenate biosynthetic enzyme polypeptide.

104.   An isolated *Bacillus* ketopantoate reductase polypeptide.

105.   An isolated *Bacillus subtilis* ketopantoate reductase polypeptide.

106.   An isolated *Bacillus* aspartate-α-decarboxylase polypeptide.

107.   An isolated *Bacillus subtilis* aspartate-α-decarboxylase polypeptide.

108.   An isolated nucleic acid molecule comprising a mutant *coa*X gene.

109.   An isolated nucleic acid molecule comprising a *coaX* gene.

110.   An isolated pantothenate kinase protein encoded by a *coaX* gene.

METHODS AND MICROORGANISMS FOR
PRODUCTION OF PANTO-COMPOUNDS

Abstract of the Disclosure

5   The present invention features methods of producing panto-compounds (*e.g.*, pantothenate) using microorganisms in which the pantothenate biosynthetic pathway and/or the isoleucine-valine biosynthetic pathway and/or the coenzymeA biosynthetic pathway has been manipulated. Methods featuring ketopantoate reductase overexpressing microorganisms as well as aspartate α-decarboxylase overexpressing

10   microorganisms are provided. Methods of producing panto-compounds in a precursor-independent manner and in high yield are described. Recombinant microorganisms, vectors, isolated nucleic acid molecules, genes and gene products useful in practicing the above methodologies are also provided. The present invention also features a previously unidentified microbial pantothenate kinase gene, *coaX*, as well as methods of

15   producing panto-compounds utilizing microorganisms having modified pantothenate kinase activity. Recombinant microorganisms, vectors, isolated *coaX* nucleic acid molecules and purified CoaX proteins are featured. Also featured are methods for identifying pantothenate kinase modulators utilizing the recombinant microorganisms and/or purified CoaX proteins of the present invention.

20

FIG.1
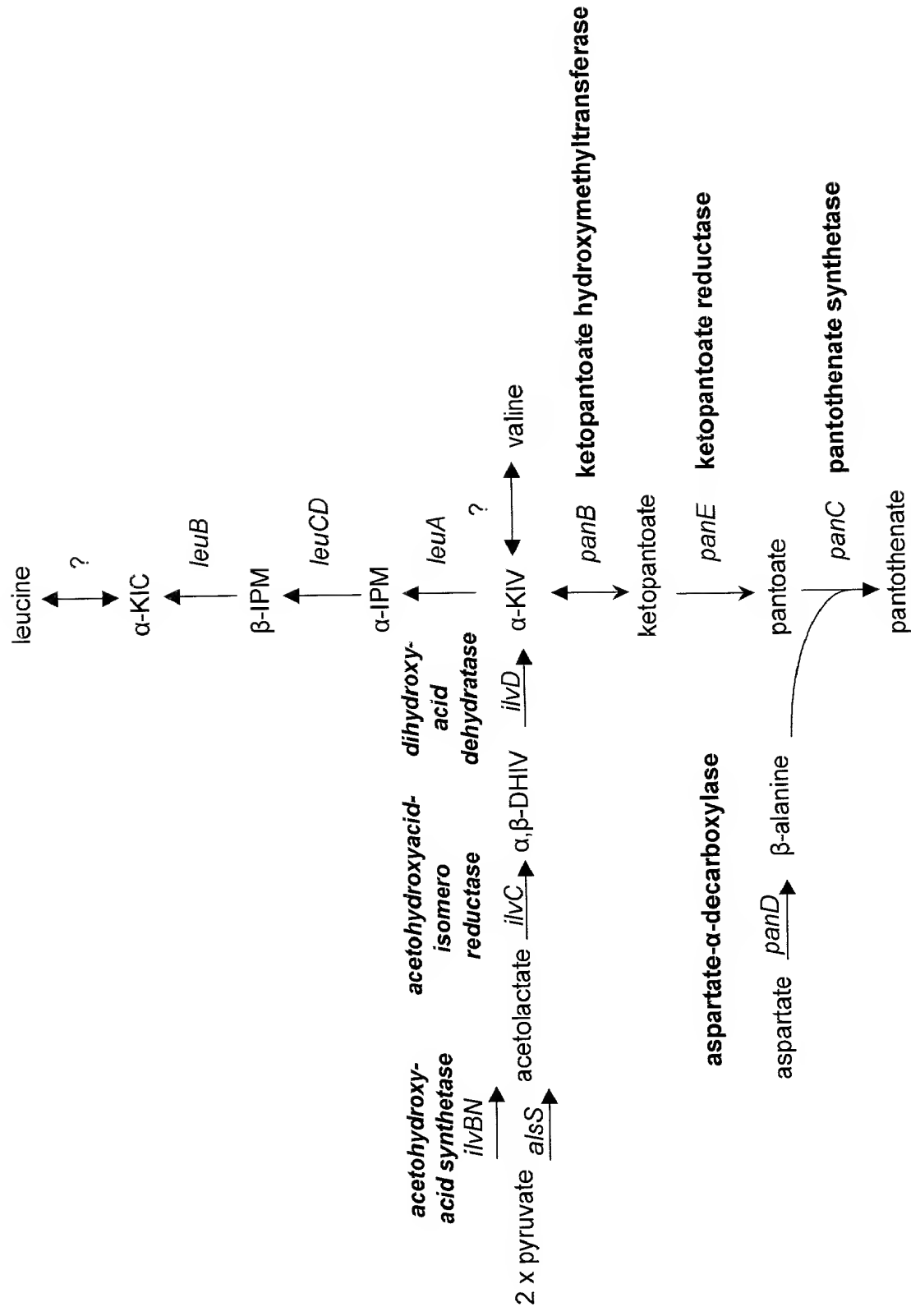
Figure 2. Plasmid pAN240, containing sequences ligated upstream of the P$_{26}$panBCD cassette, equivalent to the integrated version in strain PA221.
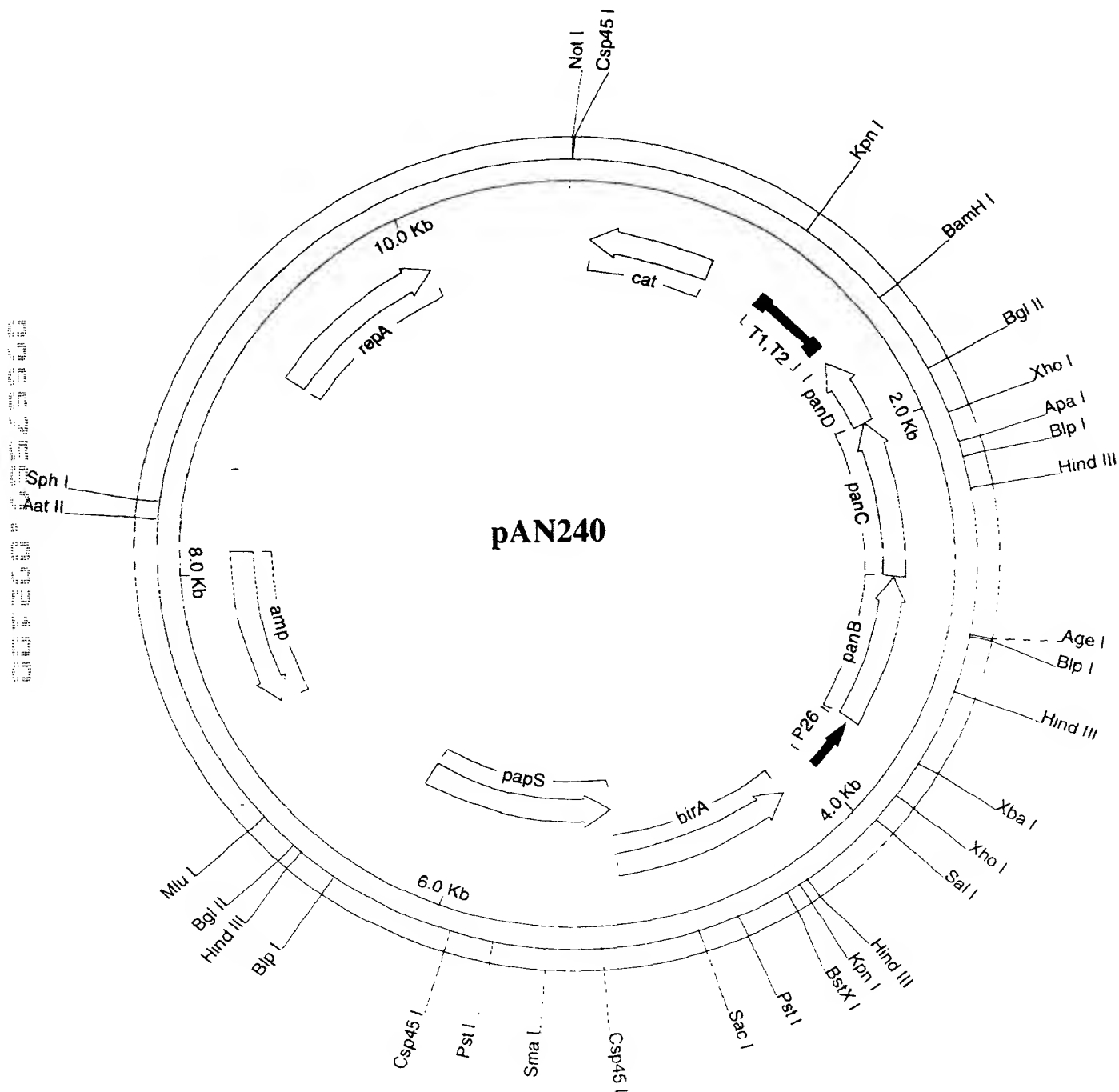
Figure 3A Plasmid pAN004, containing the panBCD *operon expressed from P26 and RBS1.*
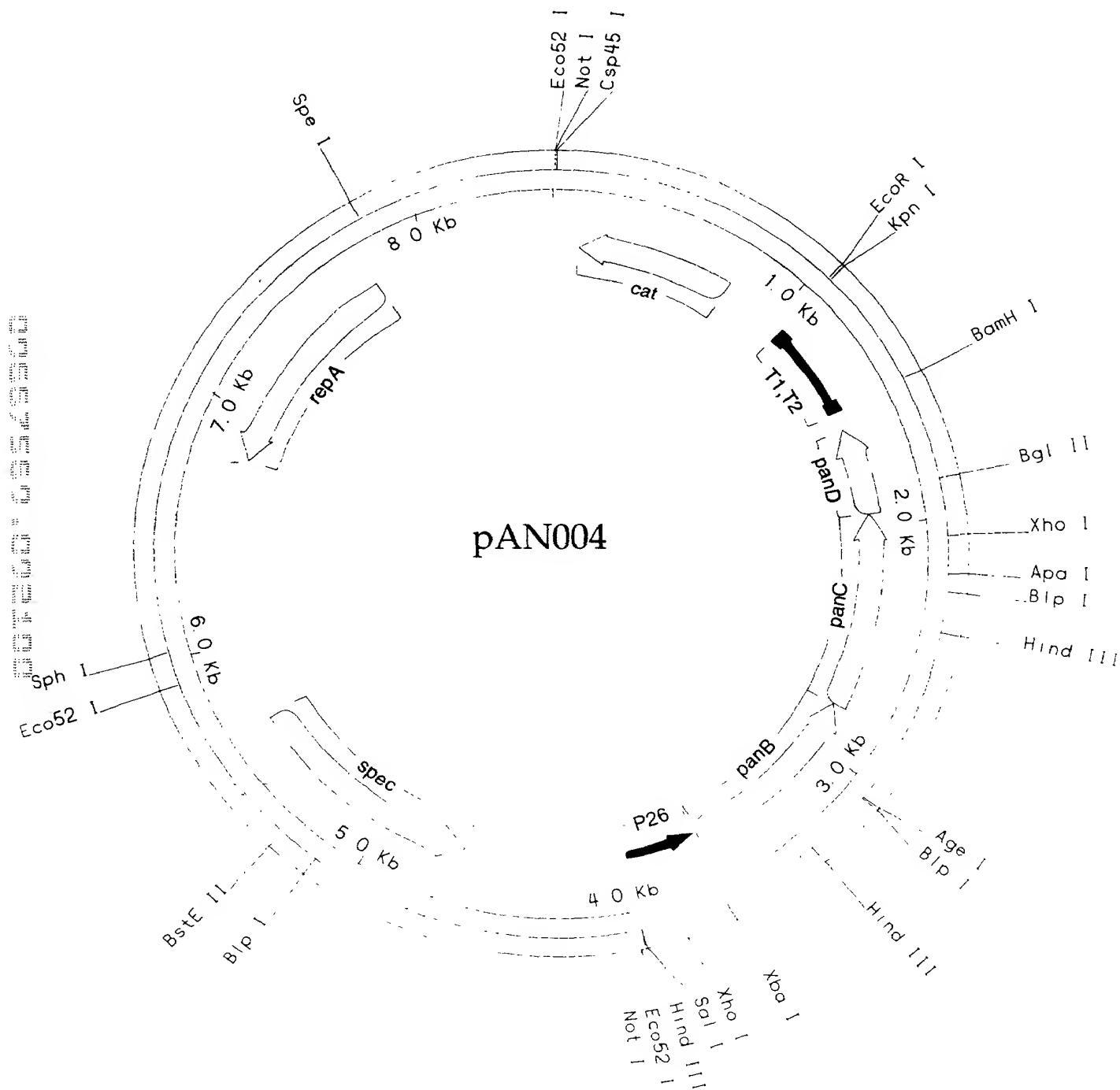
Figure 3β *Plasmid pAN006, containing the* panBCD *operon expressed from P26 and RBS2.*
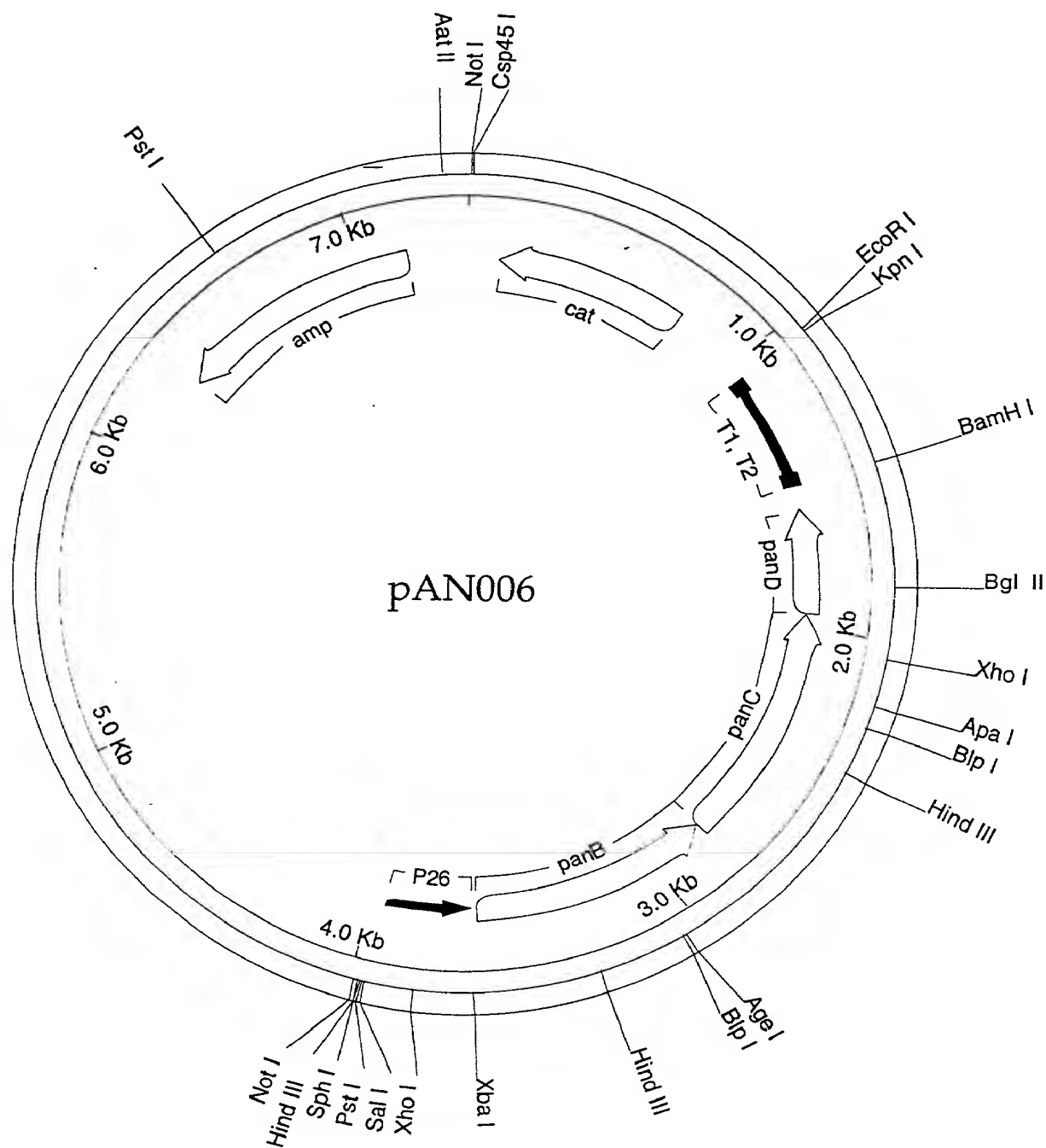
Figure 4 **Plasmid pAN236, containing an integratable and amplifiable P26-RBS2-panE1 expression cassette.**

Figure 5 *Construction of pAN423*
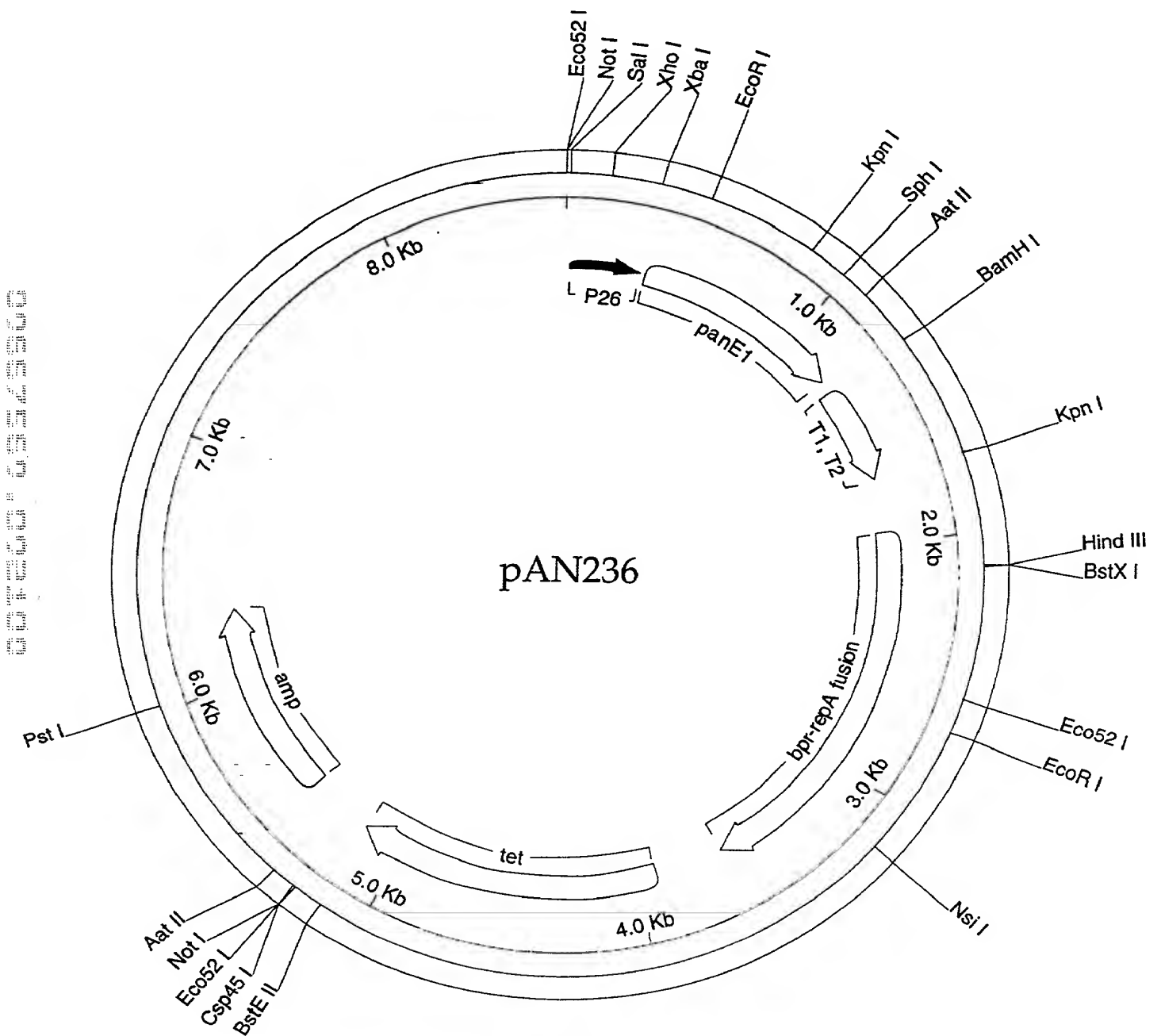
Figure 5 Construction of pAN423
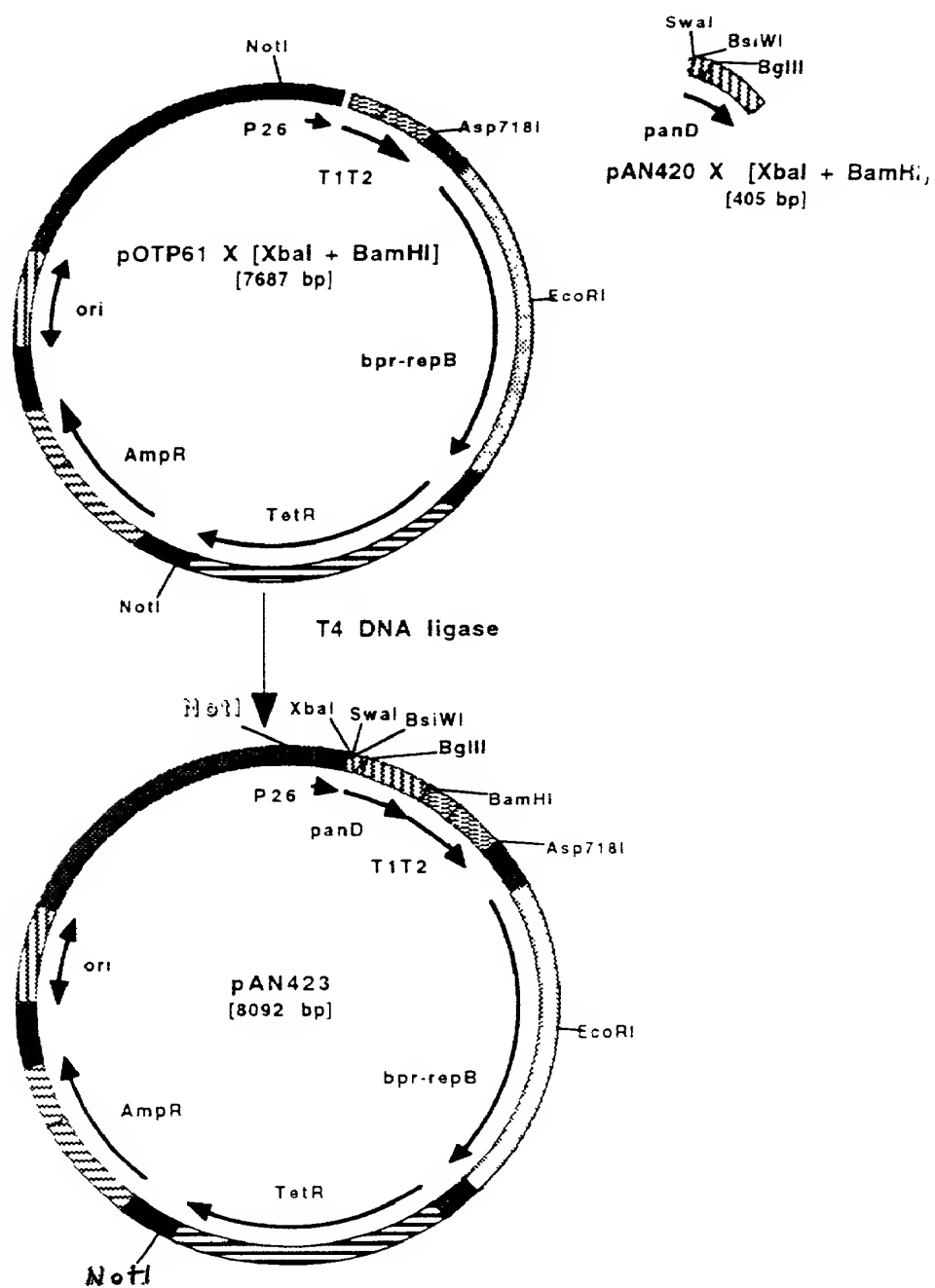
Figure 6 Construction of pAN426 and pAN427.

pAN420 X (NsiI + XbaI)
[1161 bp]

ori

BamHI
SmaI
BamHI

f1-IG

pAN420 X (NsiI + BsiWI)
[2393 bp]

AmpR

+

Swal
CTAGATTAGAAAGGAGGATTTAAAT ATG TAT CGT AC
TAATCTTTCCTCCTAAATTTA TAC ATA GCA TG-

NDA oligo cassette

PacI
CTAGATTAGAAAGGAGGTTTAATTA ATG TAT CGT AC
TAATCTTTCCTCCAAATTAAT TAC ATA GCA TG-

NDB oligo cassette

XbaI SwaI
BsiWI

panD

BamHI
SmaI
BamHI

ori

pAN426
[3588 bp]

f1-IG

Tet
Repressor

NsiI

AmpR

XbaI PacI
BsiWI

panD

BamHI
SmaI
BamHI

ori

pAN427
[3588 bp]

f1-IG

Tet
Repressor

NsiI

AmpR

Figure 7 *Construction of pAN428 and pAN429.*

Figure 8. Construction of pAN431.
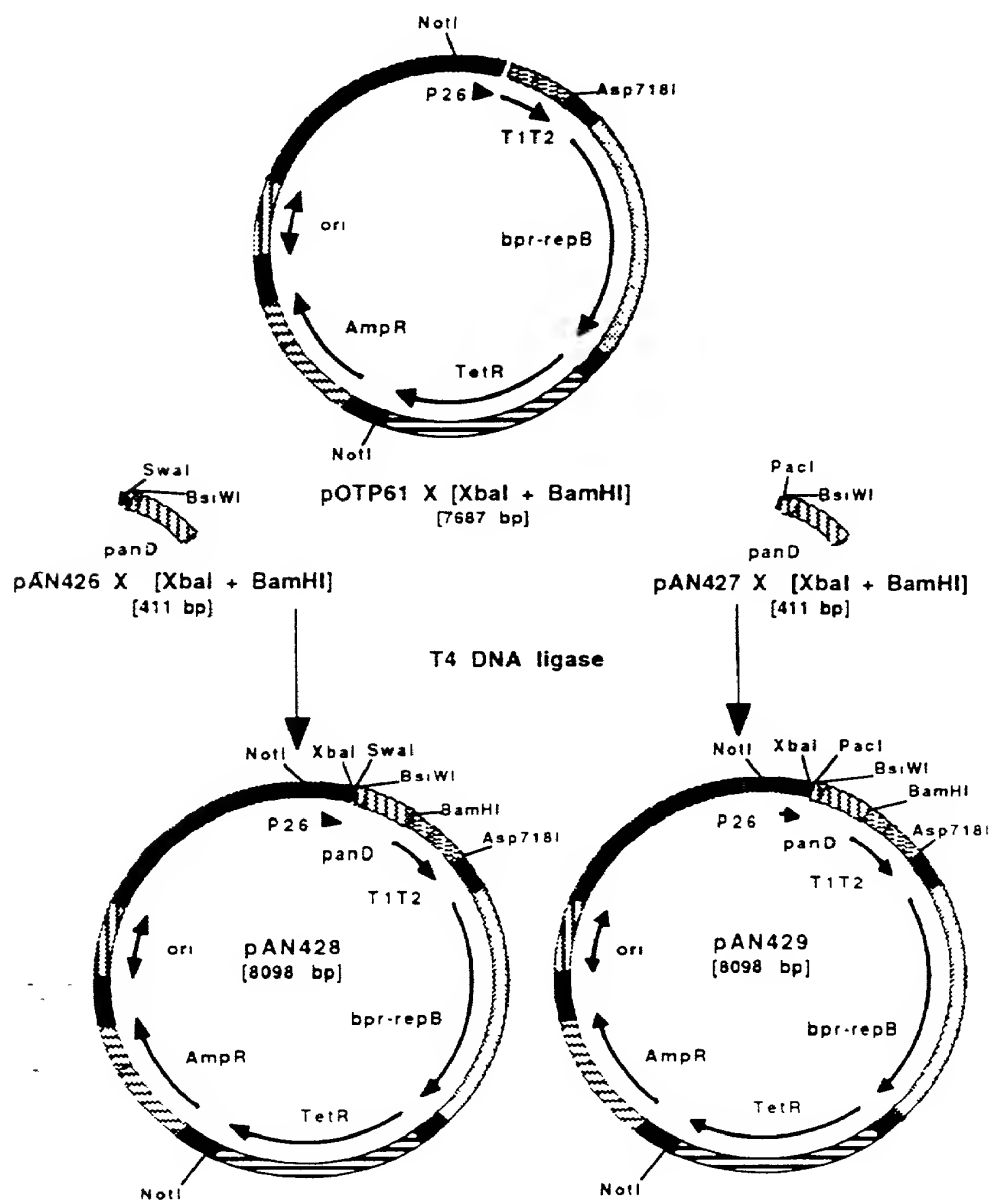
Figure 8. Construction of pAN431.
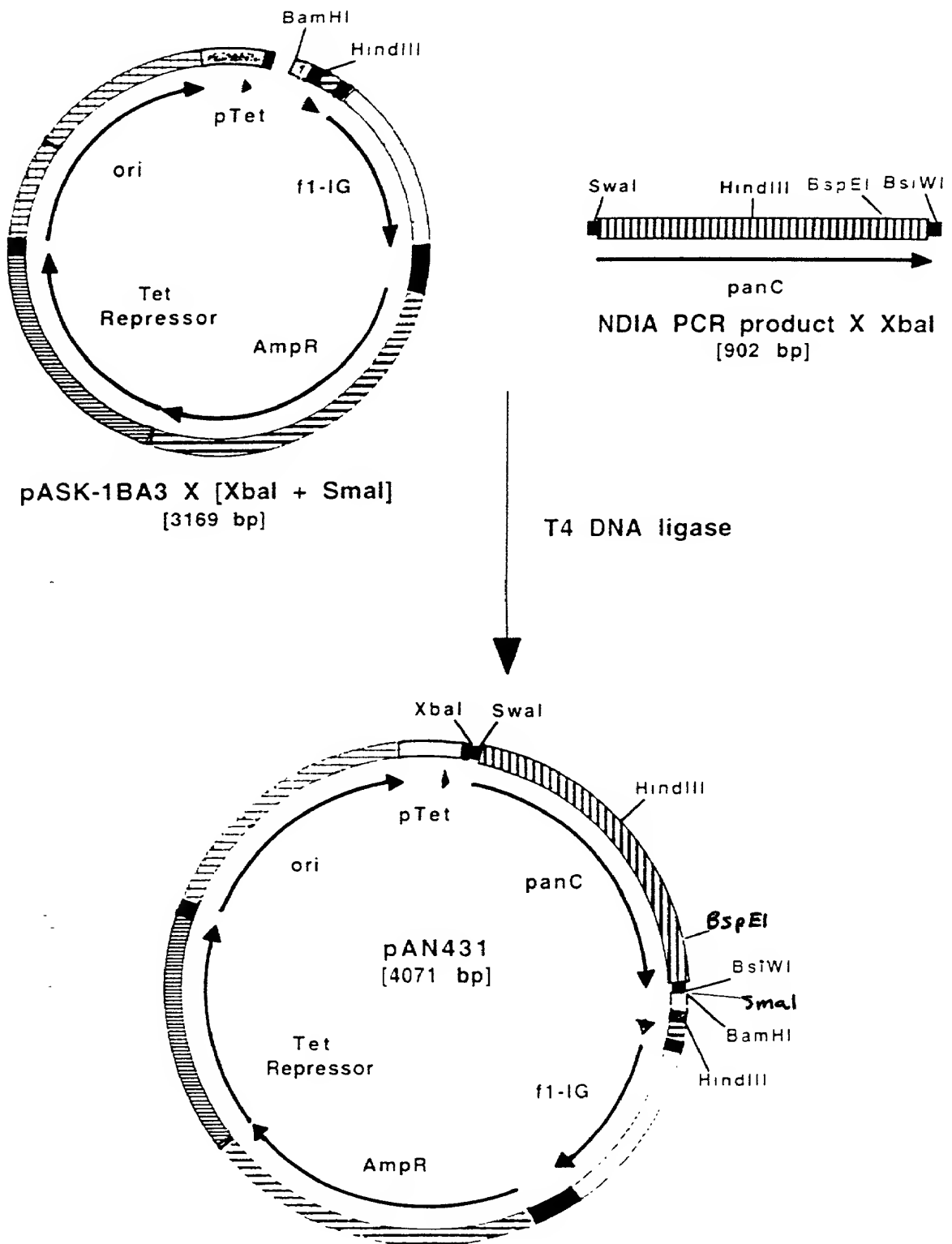
Figure 9. Construction of pAN441.

pAN431 X [Nsil + BsiWI]
[2055 bp]

Xbal   Swal
HindIII
pTet
ori
panC
BspEI

BglII
BamHI
SmaI
'panD
BamHI
HindIII
f1-IG
AmpR

pAN420 X [Nsil + BsiWI]
[2395 bp]

T4 DNA ligase

Xbal   Swal
HindIII
pTet
ori   panC
BspEI
pAN441
[4450 bp]
BsiWI
BglII
Nsil
panD
BamHI
Tet
repressor
SmaI
f1-IG
BamHI
AmpR
HindIII
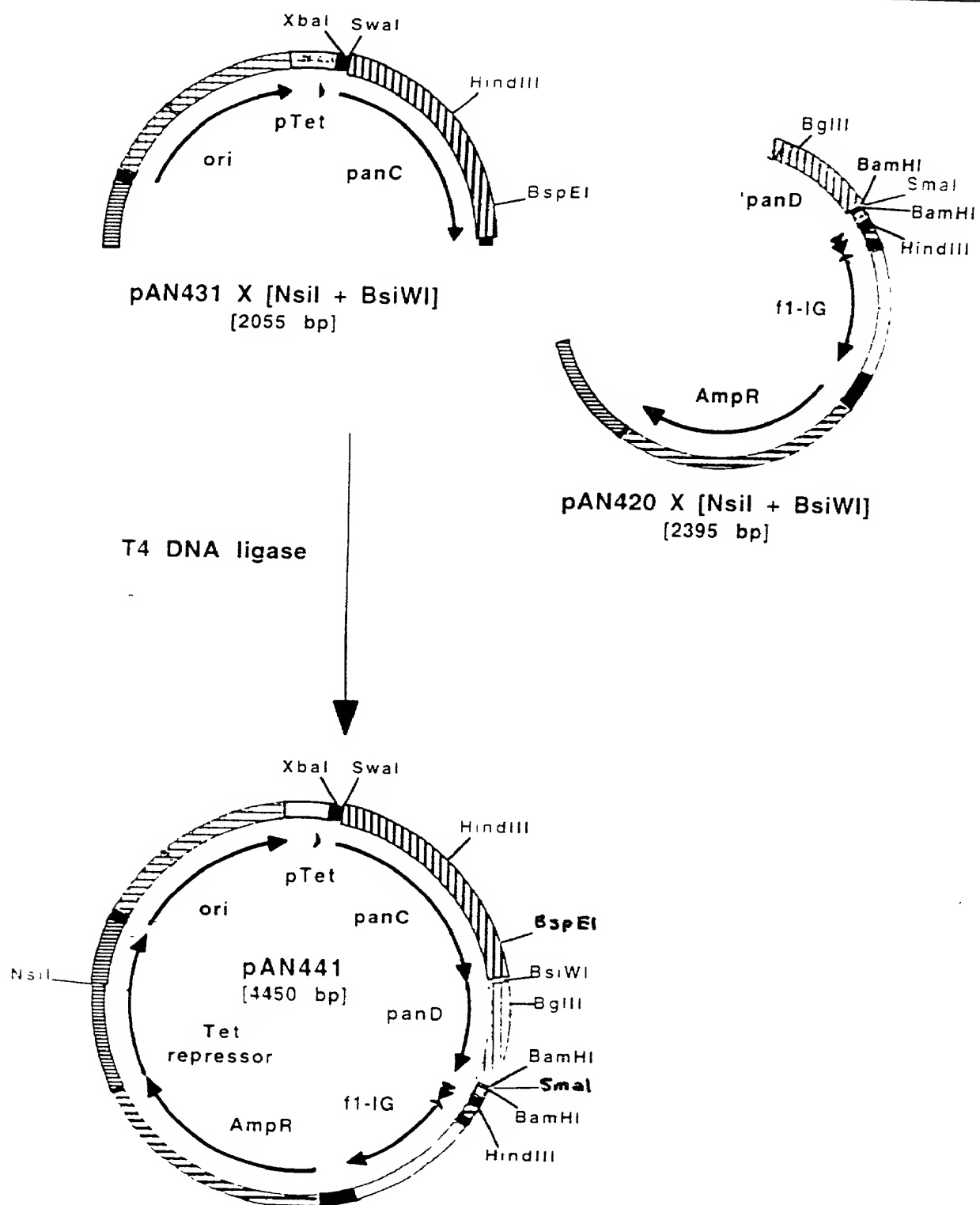
Figure 10. Construction of pAN440.

Figure 10. Construction of pAN440.

pAN430 X [BspE1 + BgIII]
[4235 bp]

pECC4 X [Aval + BamHI]
[1586 bp]

T4 DNA ligase

pAN440
[5821 bp]
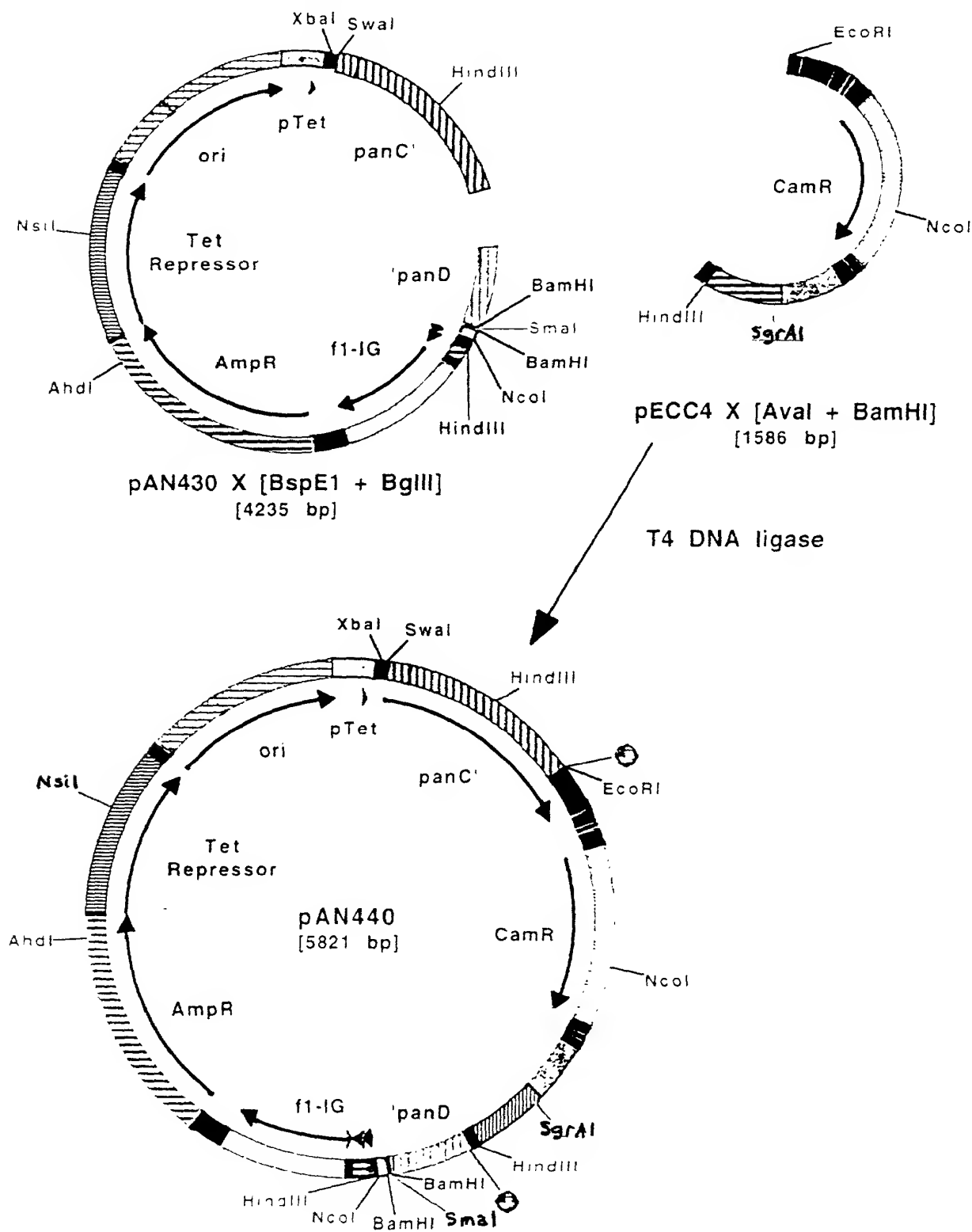
Figure 11 *Structure of pAN251, a plasmid designed to integrate a single copy of* P_{26} *panE1 at the panE1 locus by double crossover.*
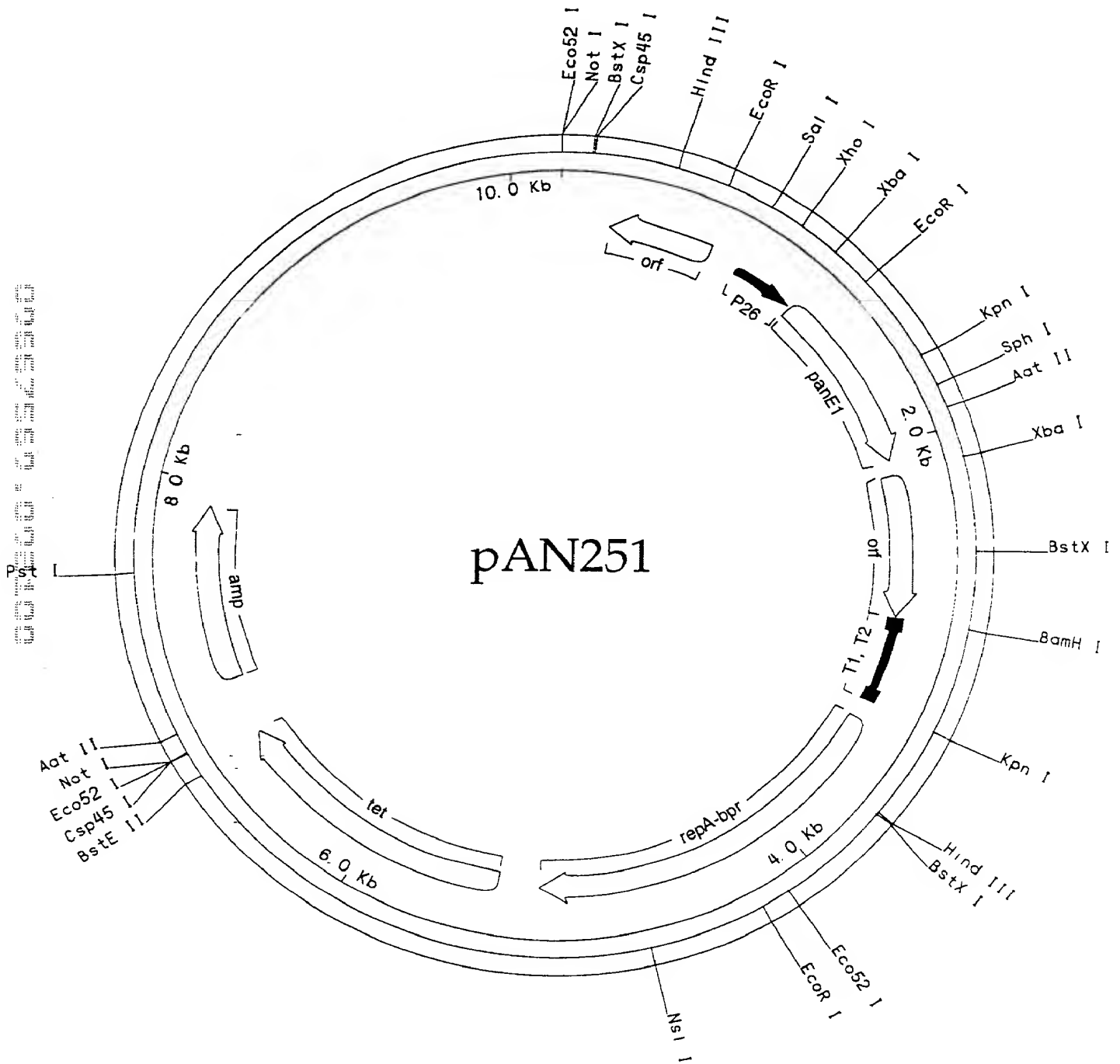
Figure 12. *Structure of pAN267, a plasmid designed to stably integrate a* P$_{26}$ *ilvBNC cassette at the* **amyE** *locus.*
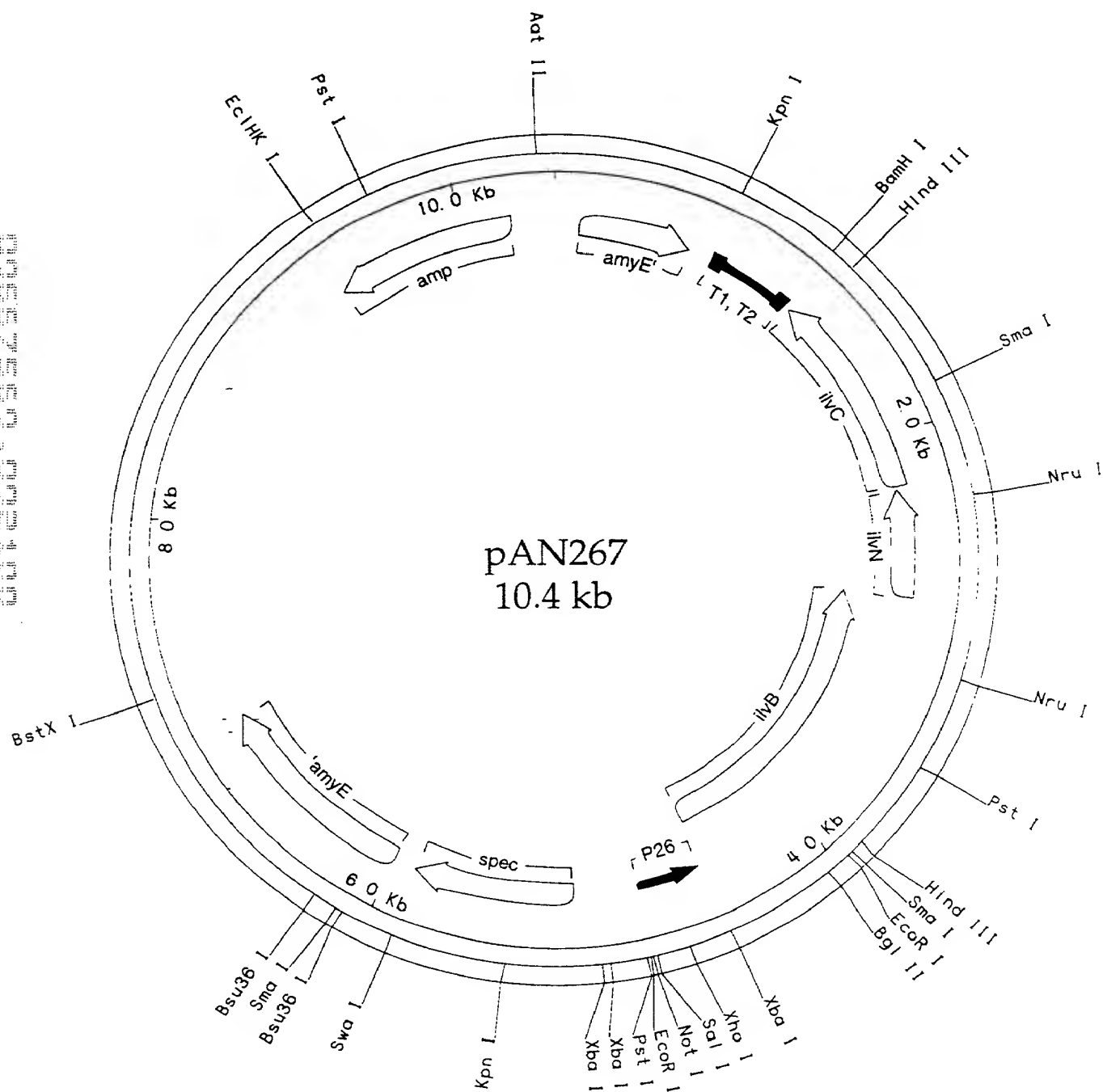
Figure 13 Structure of pAN257, a clone of B. subtilis ilvD in a low copy vector.
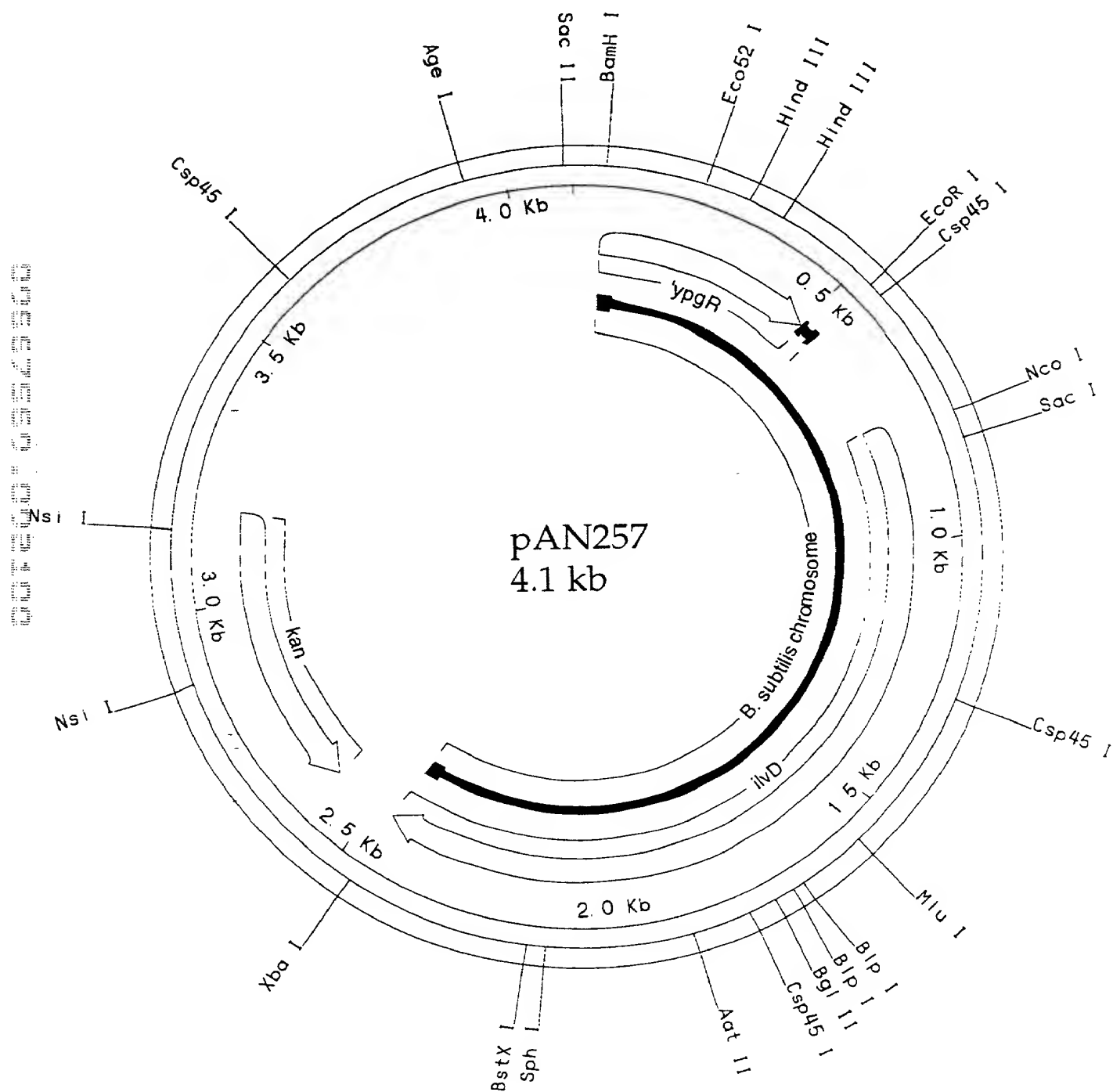
Figure 14 *Structure of pAN263, designed to stably integrate a single copy of* P_{26} ilvD *at the ilvD locus.*

Csp45 I

Age I

Sac II

BamH I

Eco52 I

Hind III

Hind III

Sal I

Xho I

Xba I

Nco I

Sac I

Csp45 I

Mlu I

Blp I

Blp I

Bgl II

Csp45 I

Aat II

Sph I

BstX I

Xba I

Nsi I

Nsi I

4.0 Kb

3.5 Kb

3.0 Kb

2.5 Kb

2.0 Kb

1.5 Kb

1.0 Kb

0.5 Kb

'ypgR

P26-RBS2

kan

ilvD

pAN263
4.2 kb

pAN261
4.0 kb

Figure 16

pantothenate

ATP ⟶
pantothenate kinase
(*coaA, panK, coaX*)
ADP ⟵

4'-phosphopantothenate

CTP, cysteine ⟶

4'-phosphopantothenylcysteine

CO_2 ⟵

4'-phosphopantetheine

ATP ⟶
coaD
PP_i ⟵

Dephosphocoenzyme A

ATP ⟶

ADP ⟵

Coenzyme A

apo-ACP

Holo-ACP
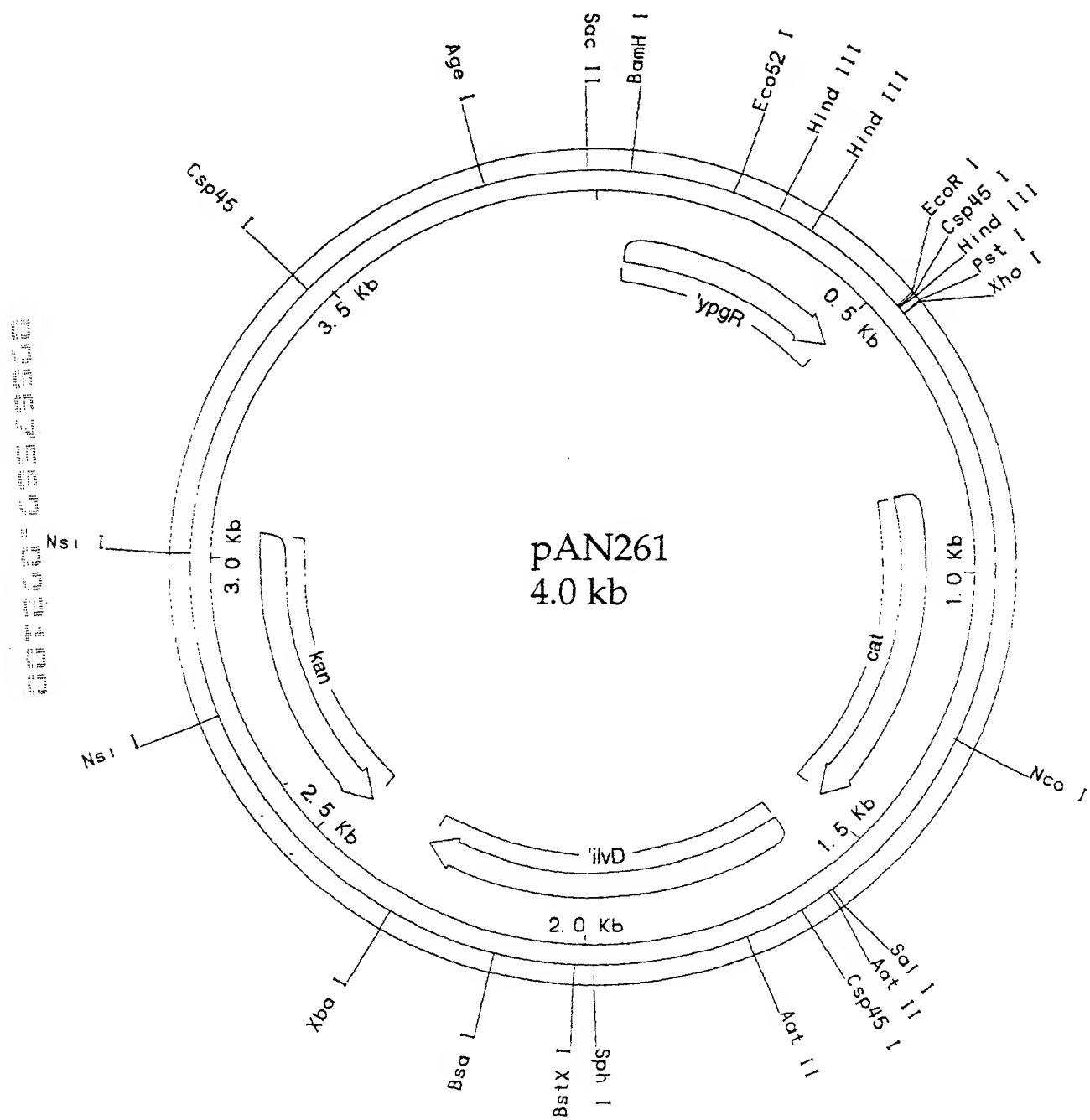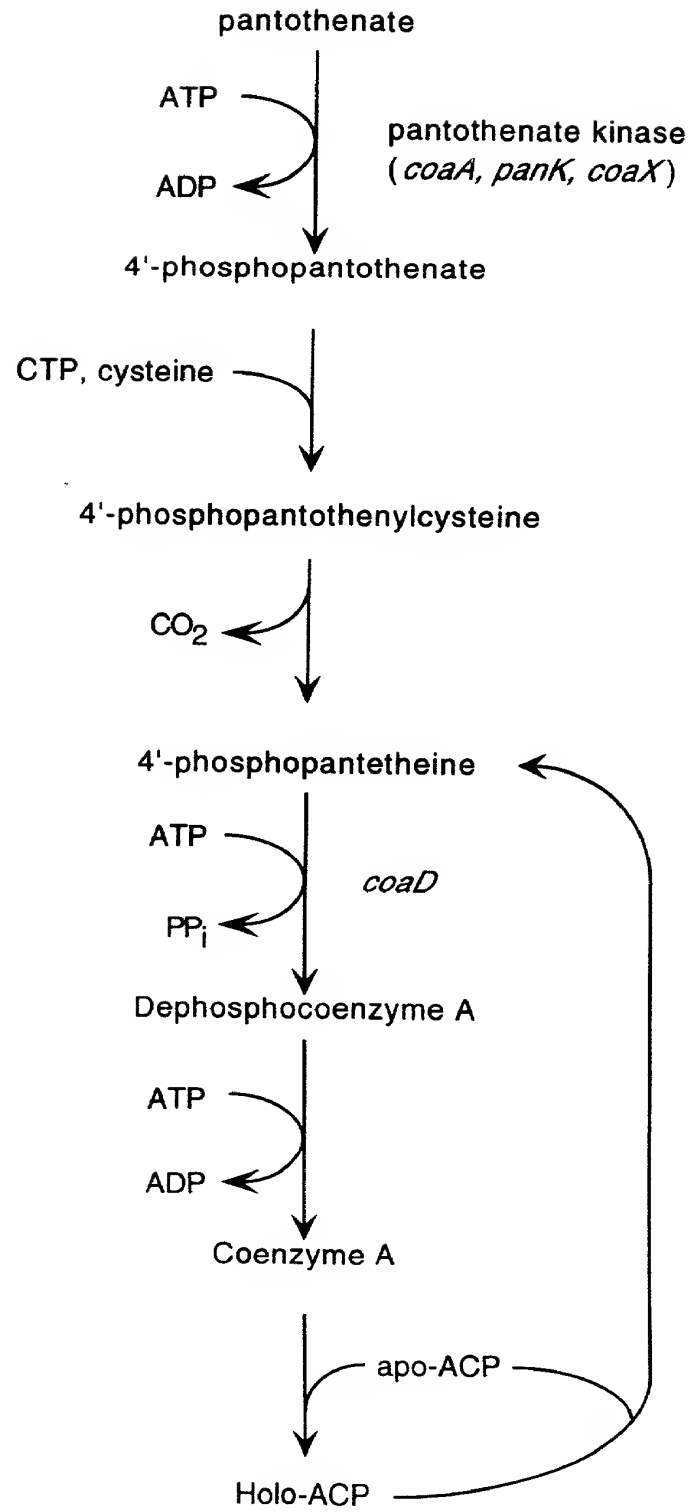
Figure 17 Structure of pAN296, designed to delete most of the B. subtilis coaA gene and substitute a chloramphenicol resistance gene.
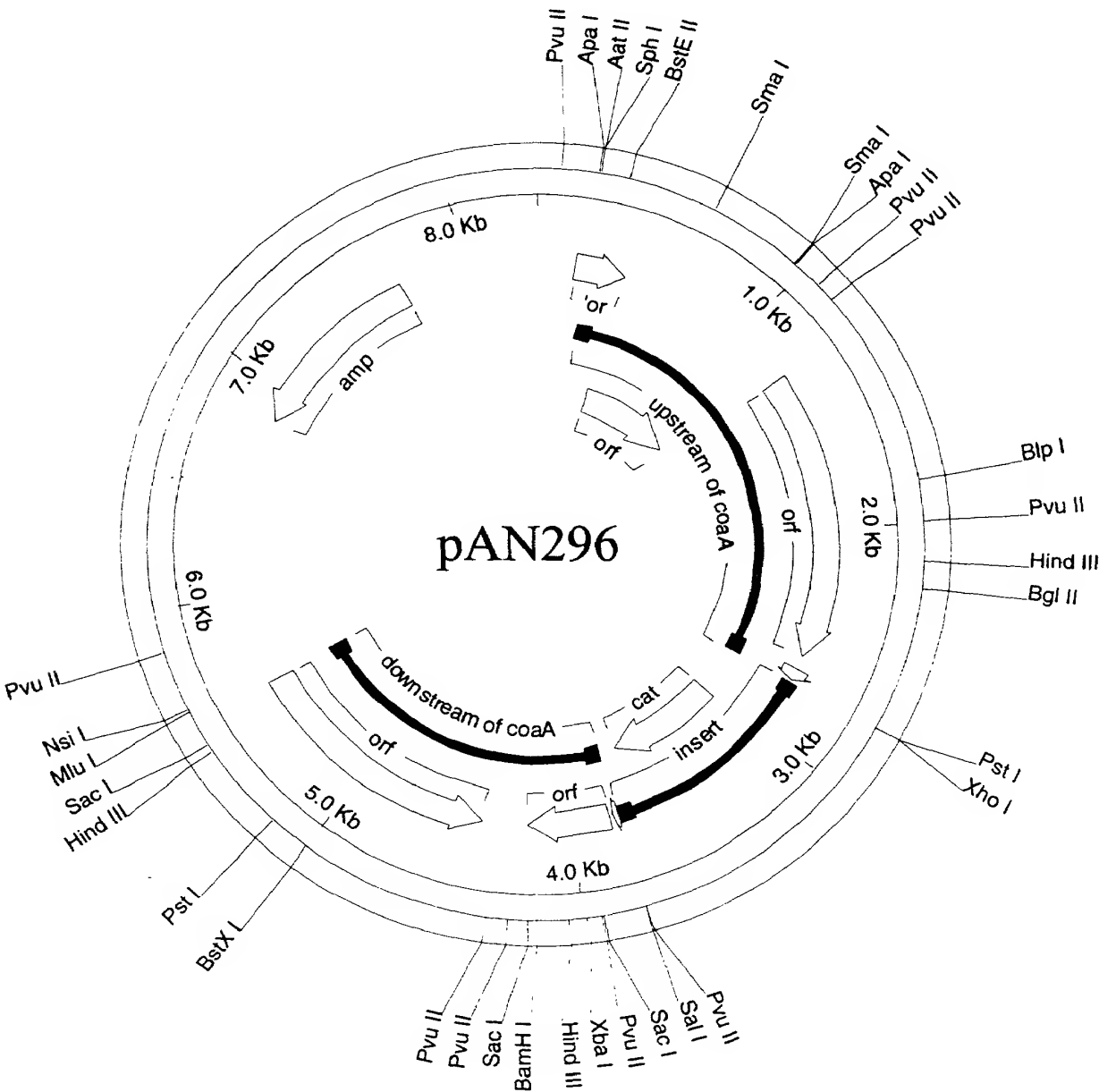
Figure 18 Structure of the B. subtilis chromosome in the region of the coaA gene. The scale is in base pairs and the significant open reading frames are shown by the open arrows.
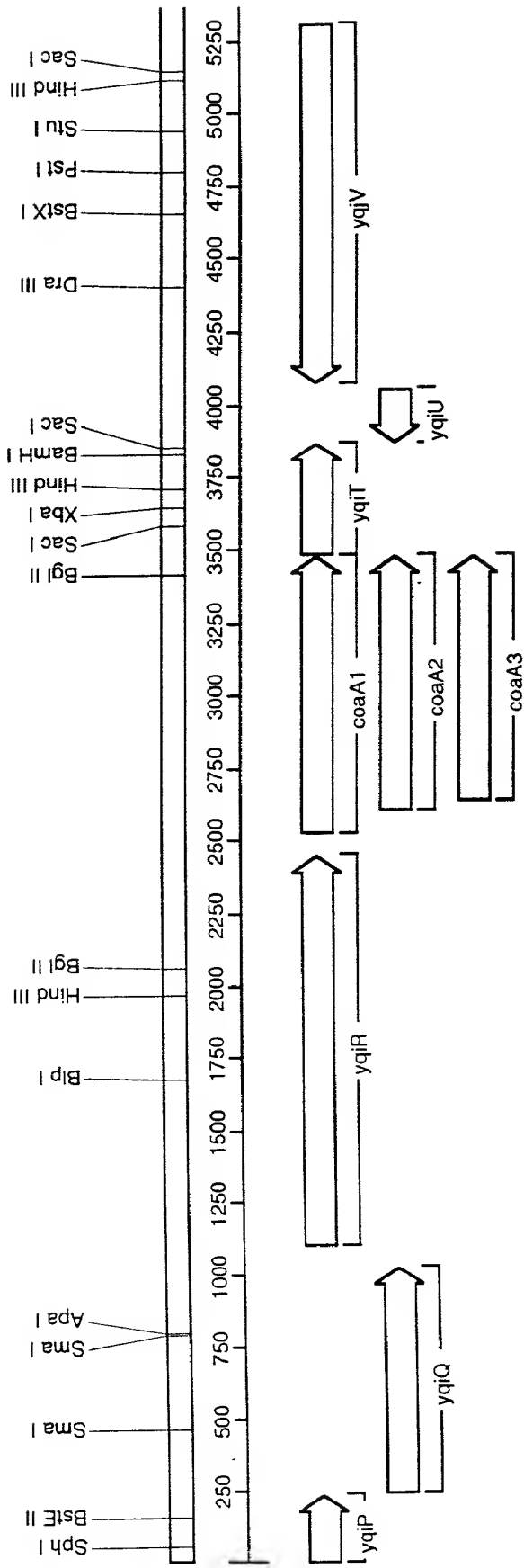
Figure 19 Structure of pAN281, a plasmid for expressing B. subtilis coaA after integration at the bpr locus. pAN282 and pAN283 have similar structures.

FIG.20A

CLUSTAL W (1.7) Multiple Sequence Alignments

Sequence type explicitly set to Protein
Sequence format is Pearson
Sequence 1: sp|Q9X795|M.leprae   312 aa
Sequence 2: sp|O86779|S.coelicolor   329 aa
Sequence 3: sp|O53440|M.tuberculosis   312 aa
Sequence 4: sp|P54556|B.subtilis   319 aa
Sequence 5: sp|P44793|H.influenzae   311 aa
Sequence 6: sp|P15044|E.coli   316 aa

```
sp|Q9X795|M.leprae        ------MPRLSE---P--SPYVEFDRKQWRALRMSTPIALTEELIGLR
sp|O53440|M.tuberculosis  ------MSRLSE---P--SPYVEFDRRQWRALRMSTPIALTEELVGLR
sp|O86779|S.coelicolor    MISPVPSIPRSAHRQRPEATPVDLTRPEWSALRDKTPLPLTAEEVEKLR
sp|P44793|H.influenzae    ----MEFSTQ------QTPFLSFNREQWAELRKSVPLKLTEQDLKPLL
sp|P15044|E.coli          ----MSIKEQTL-----MTPYLQFDRNQWAALRDSVPMTLSEDEIARLK
sp|P54556|B.subtilis      ------MKNKELN---LHTLYTQHNRESWSGFGGHLSIAVSEEEAKAVE
                                .        :  : :   .  .  :   .  :: :: ::  .
```

```
sp|Q9X795|M.leprae        GLGEQIDLLEVEEVYLPLARLIHLQVAARQRLFAATAEFLGEPQQNPGRP
sp|O53440|M.tuberculosis  GLGEQIDLLEVEEVYLPLARLIHLQVAARQRLFAATAEFLGEPQQNPDRP
sp|O86779|S.coelicolor    GLGDVIDLDEVRDIYLPLSRLLNLYVGATDGLRGALNTFLGE--QGSQSG
sp|P44793|H.influenzae    GFNEDLSLDEVSTIYLPLTRLINYYIDENLHRQTVLHRFLGR---NNAK
sp|P15044|E.coli          GINEDLSLEEVAEIYLPLSRLLNFYISSNLRRQAVLEQFLGT----NGQR
sp|P54556|B.subtilis      GLNDYLSVEEVETIYIPLVRLLHLHVKSAAERNKHVNVFLKHP---HSAK
                          *:.: ::. :*  ** :**  :.*  **::                 **
```

```
sp|Q9X795|M.leprae        VPFIIGVAGSVAVGKSTTARVLQALLARWDHHTRVDLVTTDGFLYPNAEL
sp|O53440|M.tuberculosis  VPFIIGVAGSVAVGKSTTARVLQALLARWDHHPRVDLVTTDGFLYPNAEL
sp|O86779|S.coelicolor    TPFVIGVAGSVAVGKSTVARLLQALLSRWPEHPRVELVTTDGFLLPTREL
sp|P44793|H.influenzae    TPYIISIAGSVAVGKSTSARILQSLLSHWPTERKVDLITTDGFLYPLNKL
sp|P15044|E.coli          IPYIISIAGSVAVGKSTTARVLQALLSRWPEHRRVELITTDGFLHPNQVL
sp|P54556|B.subtilis      IPFIIGIAGSVAVGKSTTARILQKLLSRLPDRPKVSLITTDGFLFPTAEL
                          *.::*..:********* **:** :**::.   . .*.*:.******* *
```

FIG.20B

sp|Q9X795|M.leprae　　　　　GRRNLMHRKGFPESYNRRALMRFVTSVKSGADYACAPVYSHLRYDTIPGA
sp|O53440|M.tuberculosis　QRRNLMHRKGFPESYNRRALMRFVTSVKSGSDYACAPVYSHLHYDIIPGA
sp|O86779|S.coelicolor　　EARGLMSRKGFPESYDRRALTRFVADIKAGKAEVTAPVYSHLIYDIVPDQ
sp|P44793|H.influenzae　　KQDNLLQKKGFPVSYDTPKLIRFLADVKSGKSNVTAPIYSHLTYDIIPDK
sp|P15044|E.coli　　　　　 KERGLMKKGFPESYDMHRLVKFVSDLKSGVPNVTAPVYSHLIYDVIPDG
sp|P54556|B.subtilis　　　KKKNMMSRKGFPESYDVKALLEFLNDLKSGKDSVKAPVYSHLTYDREEGV
　　　　　　　　　　　　　　　　.:: :****:**:　* .*: .:.:*: .　**:.****　**　.

sp|Q9X795|M.leprae　　　　　KHVVRHPDILILEGLNVLQTGP--------TLMVSDLFDFSLYVDARIQD
sp|O53440|M.tuberculosis　EQVVRHPDILILEGLNVLQTGP--------TLMVSDLFDFSLYVDARIED
sp|O86779|S.coelicolor　　RLVVRRPDILIVEGLNVLQPALPGKDGRT-RVGLADYFDFSVYVDARTED
sp|P44793|H.influenzae　　FDVVDKPDILILEGLNVLQTGNNK----TD-QTFVSDFVDFSIYVDAEEKL
sp|P15044|E.coli　　　　　 DKTVVQPDILILEGLNVLQSGMDYPHDPH-HVFVSDFVDFSIYVDAPEDL
sp|P54556|B.subtilis　　　FEVVEQADIVIIEGINVLQSPTLEDDRENPRIFVSDFFDFSIYVDAEESR
　　　　　　　　　　　　　　　　. * .*.**:.:**;:***.　　　　　.　　::* .***:.***.

sp|Q9X795|M.leprae　　　　　IEQWYVSRFLAMRGTAFADPESHFHHYSALTDSKAIIAAREIWRSINRPN
sp|O53440|M.tuberculosis　IEQWYVSRFLAMRTTAFADPESHFHHYAAFSDSQAVVAAREIWRTINRPN
sp|O86779|S.coelicolor　　IERWYLNRFRKLRATAFQNPSSYFRKYTQVSEEEALDYARTTWRTINKPN
sp|P44793|H.influenzae　　LKEWYIKRFLKFRESAFNDPNSYFKHYASLSKEEAIATASKIWDEINGLN
sp|P15044|E.coli　　　　　 LQTWYINRFLKFREGAFTDPDSYFHNYAKLTKEEAIKTAMTLWKEINWLN
sp|P54556|B.subtilis　　　IFTWYLERFRLLRETAFQNPDSYFHKFKDLSDQEADEMAASIWESVNRPN
　　　　　　　　　　　　　　　　: **:.** .*. :* .* :*.*;*::::　.:::..;**　*　.* :*. :*.

sp|Q9X795|M.leprae　　　　　LVENILPTRPRATLVLRKDADHSINRLRLRKL
sp|O53440|M.tuberculosis　LVENILPTRPRATLVLRKDADHSINRLRLRKL
sp|O86779|S.coelicolor　　LVENVAPTRGRATLVLRKGPDHKVQRLSLRKL
sp|P44793|H.influenzae　　LNQNILPTRERANLILKKGHNHQVELIKLRK-
sp|P15044|E.coli　　　　　 LKQNILPTRERASLILTKSANHAVEEVRLRK-
sp|P54556|B.subtilis　　　LYENILPTKFRSDLILRKGDGHKVEEVLVRRV
　　　　　　　　　　　　　　　　* .*:.**: *: *.* *　.* .*　.: .* ::

Figure 21
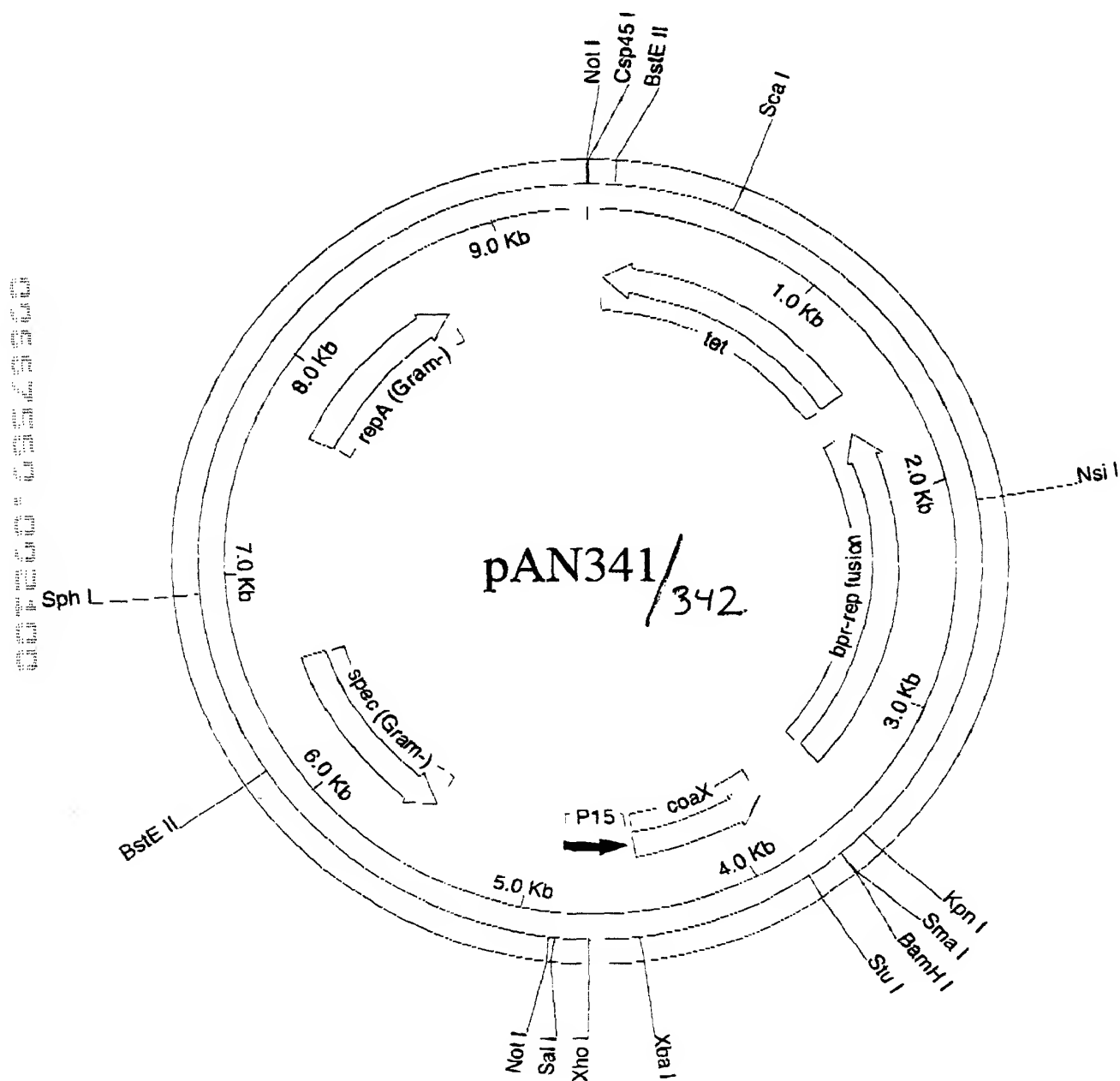
FIG.23A

CLUSTAL W (1.7) Multiple Sequence Alignments

Sequence type explicitly set to Protein
Sequence format is Pearson

```
Seq. 1: B.subtilis|CoaX|SEQNO_9        258 aa
Seq.2: dbj|BAA21476.1|D.vulgaris       212 aa
Seq.3: gb|AAD35964.1|T.maritima        246 aa
Seq.4: pir|T36391|S.coelicolor         265 aa
Seq.5: sp|Q45338|B.pertussis           267 aa
Seq.6: sp|O06282|M.tuberculosis        272 aa
Seq.7: sp|O83446|T.pallidum            273 aa

Seq.8:  sp|O51477|B.burgdorferi            262 aa
Seq.9:  sp|P74045|Synechocystis           257 aa
Seq.10: sp|O25533|H.pylori               223 aa
Seq.11: sp|O67753|A.aeolicus             229 aa
Seq.12: sp|Q9RX54|D.radiodurans          262 aa
Seq.13: WIT|RCA03301|C.acetobutylicum    250 aa
Seq.14: WIT|RRC02473|R.capsulatus        258 aa
```

```
B.subtilis|CoaX|SEQIDNO_9          --------------MLLVIDVGNTNTVLGVYHDG-----KLEYHWRIE
WIT|RCA03301|C.acetobutylicum      NKRAAFMLLFLRSVLKVILVLDVGNTNIVLGIYNDT----KLTAEWRLS
pir|T36391|S.coelicolor            --------------MLLTIDVGNTHTVLGLFDGE----DIVEHWRIS
sp|O06282|M.tuberculosis           --------------MLLAIDVRNTHTVVGLLSGMKEHAKVVQQWRIR
WIT|RRC02473|R.capsulatus          --------------MLLCIDCGNTNTVFSVWDGT----DFAATWRIA
dbj|BAA21476.1|D.vulgaris          -------------MTQHFLLFDIGNTNVKIGIAVET----AVLTSYVLP
sp|Q9RX54|D.radiodurans            -----------MPAFPLLAVDIGNTTVLGLADASG----ALTHTWRIR
gb|AAD35964.1|T.maritima           -----------MYLLVDVGNTHSVFSITEDG----KTFRRWRLS
sp|O83446|T.pallidum               ------------MLLIDVGNSHVVFGIQGENGGRVCVRELFRLA
sp|O51477|B.burgdorferi            -----------MNKPLLSELIIDIGNTSIAFALFKDN----QVNLFIKMK
sp|O67753|A.aeolicus               ----------MRFLTVDVGNSSVDIALWEGK--------KVK
sp|P74045|Synechocystis            ---------METSKPGCGLALDNDKQKPWLGLMIGN----SRLHWAYC
sp|O25533|H.pylori                 ----------MPARQSFTDLKN----LVLCDIGN-----------TR
sp|Q45338|B.pertussis              --------------MILIDSGNSRLKVGWFDPDAP---QAAREPAPV

                                                 *                          :   :
```

B.subtilis|CoaX|SEQIDNO_9   TSRHKTEDEFGMILRSLFDHS-----GLMFEQIDGIISSVVPPIMFALER
WIT|RCA03301|C.acetobutylicum   TDVLRSADEYGIQVMNLFQQD----KLDPTLVEGVIISSVVPNIMYSLEH
pir|T36391|S.coelicolor   TDSRRTADELAVLLQGLMGMHPLLGDELGDGIDGIAICATVPSVLHELRE
sp|O06282|M.tuberculosis   TESEVTADELALTIDGLIG------EDSERLTGTAALSTVPSVLHEVRI
WIT|RRC02473|R.capsulatus   TDHRRTADEYFVWLNTLMQLK-----GLQGRISEAIISSTAPRVVFNLRV
dbj|BAA21476.1|D.vulgaris   TDPGQTTDSIGLRLLEVLRHAG----LGPADVGACVASSVVPGVNPLIRR
sp|Q9RX54|D.radiodurans   TNREMLPDDLALQLHGLFTLA------GAP-IPRAAVLSSVAPPVGENYAL
gb|AAD35964.1|T.maritima   TGVFQTEDELFSHLHPLLG-------DAMREIKGIGVASVVPTQNTVIER
sp|O83446|T.pallidum   PDARKTQDEYSLLIHALCERAG----VGRASLRDAFISSVVPVLTKTIAD
sp|O51477|B.burgdorferi   TNLMLRYDEVYSFFEENFDFN------VN---K--VFISSVVPILNETFKN
sp|O67753|A.aeolicus   DFLKLSHEEFLKEEFPKLK------------------ALGISVKQSFSEKVRG
sp|P74045|Synechocystis   SGNAPLQTWVTDYNPKSAQLP----------------VLLGKVPLMLASVVPE
sp|O25533|H.pylori   IHFAQNYQLFSSAKEDLKR--------------------LGIQKEIFYISVNEE
sp|Q45338|B.pertussis   AFDNLDLDALGRWLATLPRRP---------Q------RALGVNVAGLARGEAIA

B.subtilis|CoaX|SEQIDNO_9   MCTKYFHIEPQIVG-PG-MKTGLNIKYDNPKEVGADRIVNAVAAIHLYG-
WIT|RCA03301|C.acetobutylicum   MIRKYFKINPLVVG-PG-IKTGINIKYDNPKEVGADRIVNAVAAHEIYK-
pir|T36391|S.coelicolor   VTRRYYGDVPAVLVEPG-VKTGVPILTDHPKEVGADRIINAVAAVELYG-
sp|O06282|M.tuberculosis   MLDQYWPSVPHVLIEPG-VRTGIPLLVDNPKEVGADRIVNCLAAYDRFR-
WIT|RRC02473|R.capsulatus   LCNRYFDCRPYVVGKPG-CELPVAPRVDPGTTVGPDRLVNTVAGYDRHG-
dbj|BAA21476.1|D.vulgaris   ACERYL--YRKLLFAPGDIAIPLDNRYERPAEVGADRIVAAYAARRLYP-
sp|Q9RX54|D.radiodurans   ALKRHFMIDAFAVSAEN--LPDVTVELDTPGSVGADRLCNLFGAEKYLG-
gb|AAD35964.1|T.maritima   FSQKYFHISPIWVKAKN--GCVKWNVKNPSEVGADRVANVVAFVKEYG-
sp|O83446|T.pallidum   AVAQISGVQPVVFGPWAYEHLPVRIPEPVRAEIGTDLVANAVAAYVHFR-
sp|O51477|B.burgdorferi   VIFSFFKIKPLFIGFDLNYDLTFNPYKSDKFLLGSDVFANLVAAIENYS-
sp|O67753|A.aeolicus   KIPKIK-----FLKKEN---FPIQVDYKTPETLGTDRVALAYSAKKFYG-
sp|P74045|Synechocystis   QTEVWRVYQPKILTLKN---LPLVNLYP---SFGIDRALAGLGTGLITYG-
sp|O25533|H.pylori   NEKALLNCYPNAKNIAG--FFHLETDYVG---LGIDRQMACLA---VN--
sp|Q45338|B.pertussis   ATLRAGGCDIRWLRAQP-LAMGLRNGYRNPDQLGADRWACMVGVLARQPS

FIG.23C

```
B.subtilis|CoaX|SEQIDNO_9        NP--LIVVDFGTATTYCYIDENKQYMGAIAPGITISTEALYSRAAKLPR
WIT|RCA03301|C.acetobutylicum    RS--LIIIDFGTATTFCAVRENGDYLGGAICPGIKVSSEALFEKAAKLPR
pir|T36391|S.coelicolor          GP--AIVVDFGTATTFDAVSARGEYIGGVIAPGIEISVEALGVKGAQLRK
sp|O06282|M.tuberculosis         KA--AIVVDFGSSICVDVVSAKGEFLGGAIAPGVQVSSDAAAARSAALRR
WIT|RRC02473|R.capsulatus        GD--LIVVDFGTATTFDVVAPDGAYIGGVIAPGVNLSLEALHMAAAALPH
dbj|BAA21476.1|D.vulgaris        GPRSLVSVDFGTATTFDCVEG-GAYLGGLICPGVLSSAGALSSRTAKLPR
sp|Q9RX54|D.radiodurans          GLDYAVVVDFGTSTNFDVVGRGRRFLGGILATGAQVSADALFARAAKLPR
gb|AAD35964.1|T.maritima         KN--GIIIDMGTATTVDLVVN-GSYEGGAILPGFFMMVHSLFRGTAKLPL
sp|O83446|T.pallidum             SA--CVVVDCGTALTFTAVDGTGLIQGVAIAPGLRTAVQSLHTGTAQLPL
sp|O51477|B.burgdorferi          FEN-VLVVDLGTACTIFAVSRQDGILGGIINSGPLINFNSLLDNAYLIKK
sp|O67753|A.aeolicus             KN--VVVISAGTALVIDLVLE-GKFKGGFITLGLGKKLKILSDLAEGIPE
sp|P74045|Synechocystis          FP--CLVVDGGTALTITGFDQDKKLVGGAILPGLPGLGLQLATLGDRLAALPK
sp|O25533|H.pylori               NG--VVVDAGSAITIDLIKE-GKHLGGCILPGLAQYIHAYKKSAKILEQ
sp|Q45338|B.pertussis            VHPPLLVASFGTATTLDTIGPDNVFPGGLILPGPAMMRGALAYGTAHLPL
                                     .   *  .          .   *   : .               :


B.subtilis|CoaX|SEQIDNO_9        IEITRPDN---IIGKNTVSAMQSGILFGYVGQVEGIVKRMKWQAKQDLK-
WIT|RCA03301|C.acetobutylicum    VELIKPAY---AICKNTISSIQSGIVYRYLRQVKYLFEKLKENLPDGRRT
pir|T36391|S.coelicolor          IEVARPRS---VIGKNTVEAMQSGIVYGFAGQVDGVVNRMARELADD--P
sp|O06282|M.tuberculosis         VELARPRS---VVGKNTVECMQAGAVFGFAGLVDGLVGRIREDVSGFSVD
WIT|RRC02473|R.capsulatus        VDVTKPQG---VIGTNTVACIQSGVYWGYIGLVEGIVRQIRMERDRP---
dbj|BAA21476.1|D.vulgaris        ISLEVEEDS-PVIGRSTTTSLNHGFIFGFAAMTEGVLAA-----------
sp|Q9RX54|D.radiodurans          ITLQAPET---AIGKNTVHALQSGLVFGYAEMVDGLLRRIRAELPGE---
gb|AAD35964.1|T.maritima         VEVKPADF---VVGKDTEENIRLGVVNGSVYALEGIIGRIKEVYGDLIP--
sp|O83446|T.pallidum             VPLALPDS---VLGKDTTHAVQAGVVRGTLFVIRAMIAQCQKELGCR---
sp|O51477|B.burgdorferi          FPISTPNN---LLERTTSGSVNSGLFYQYKYLIEGVYRDIKQMYKKK---
sp|O67753|A.aeolicus             FFPEEVEI---FLGRSTRECVLGGAYRESTEFIKSTLKLWRKVFKRK---
sp|P74045|Synechocystis          LEMDQLTELPDRWALDTPSAIFSGVVYGVLGALQSYLQDWQKLFPGA---
sp|O25533|H.pylori               PFKALDSL--EVLPKSTRDAVNYGMVLSVIACIQHLAK--NQK-------
sp|Q45338|B.pertussis            ADGLVADY-----PIDTHQAIASGIAAAQAGAIVRQWLAGRQRYGQAP--
                                         *            :   *
```

FIG.23D

B.subtilis|CoaX|SEQIDNO_9	----VIATGG--------	LAPLIANES-----------DCIDIVDPFLTLKGLELI
WIT|RCA03301|C.acetobutylicum	RTSLVLATGG--------	LAKLIN----------------------
pir|T36391|S.coelicolor	DDVTVIATGG--------	LAPMVLGES-----SVIDEHEPWLTLMGLRLV
sp|O06282|M.tuberculosis	HDVAIVATGH--------	TAPLILPEL-----HTVDHYDQHLTLQGIRLIV
WIT|RRC02473|R.capsulatus	--MKVIATGG--------	LASLFDLGF-----DLFDKVEDDLTMHGLRLI
dbj|BAA21476.1|D.vulgaris	--	
sp|Q9RX54|D.radiodurans	--AVAVATGG--------	FSRTVQGIC-----QEIDYYDETLTRGLVEL
gb|AAD35964.1|T.maritima	----VVLTGG--------	QSKIVK-DM-----IKHEIFDEDLTIKGVYHF
sp|O83446|T.pallidum	--CAAVITGG--------	LSRLFS-SE-----VDFPPIDAQLTLSGLAHI
sp|O51477|B.burgdorferi	--FNLIITGG--------	NADLILSLI-----EIEFIFNIHLTVEGVRIL
sp|O67753|A.aeolicus	--FKVVITGG--------	EGKYFS--------KFGIYDPLIVHRGMRNL
sp|P74045|Synechocystis	--AMVITGG---------	DGKILHGFLKEHSPNLSVAWDDNLIFLGMAAI
sp|O25533|H.pylori	----IYLCGG--------	DAKYLSAFL-----PHSVCKERLVFDGMEIA
sp|Q45338|B.pertussis	---EIYVAGGWPEVRQEAERLLAVTGAAFGATPQPTYLDSPVLDGLAAL	

B.subtilis|CoaX|SEQIDNO_9	YERNRVGSV------------
WIT|RCA03301|C.acetobutylicum	----------------------
pir|T36391|S.coelicolor	YERNVSRM--------------
sp|O06282|M.tuberculosis	FERNLEVQRGRLKTAR------
WIT|RRC02473|R.capsulatus	FDYNKGLGA-------------
dbj|BAA21476.1|D.vulgaris	----------------------
sp|Q9RX54|D.radiodurans	WASRSEVR-------------
gb|AAD35964.1|T.maritima	CFGD-----------------
sp|O83446|T.pallidum	ARLVPTSLLPPATVSGSSGN
sp|O51477|B.burgdorferi	GNSIDFKFVN-----------
sp|O67753|A.aeolicus	LYLYHRI--------------
sp|P74045|Synechocystis	HHGDRPIC-------------
sp|O25533|H.pylori	LKKAGILECK-----------
sp|Q45338|B.pertussis	AAQGAPTA-------------

Figure 24. Alignment of a portion of the amino acid sequences of several known or suspected pantothenate kinases. The residues that are mutated in E. coli coaA15(Ts) and B. subtilis coaA from plasmid pAN282A are indicated below and above the alignment, respectively. The coordinate given in the left margin for the B. subtilis protein refers to the coaA1 open reading frame.

```
        K D N V T A P V Y S H L I Y D I I P G A   Majority
                                 H
168     K D S V K A P V Y S H L T Y D R E E G V   B. subtilis CoaA1
167     V P N V T A P V Y S H L I Y D V I P D G   E. coli CoaA
165     K S N V T A P L Y S H L T Y D I P D K     H. influenzae CoaA
169     A D Y A C A P V Y S H L R Y D T I P G A   M. leprae CoaA
169     S D Y A C A P V Y S H L H Y D I P G A     M. tuberculosis CoaA
179     K A E V T A P V Y S H L I Y D V P D Q     S. coelecolor CoaA
                                 L
```

Figure 25 Structure of pAN294, a plasmid for integrating mutagenized B. subtilis coaA at its native locus.
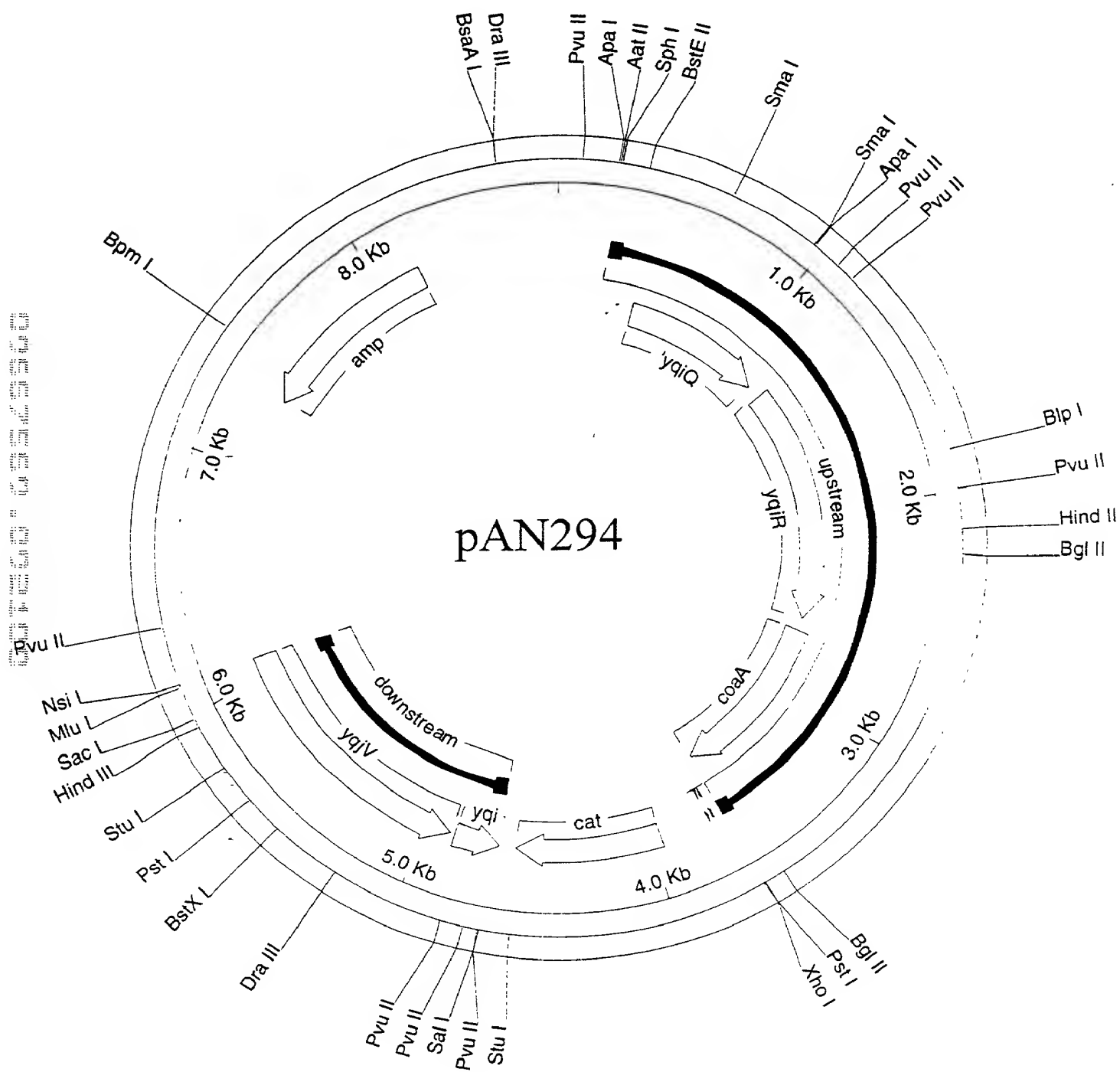
Figure 26 *Structure of pAN336, a plasmid designed to delete* B. subtilis coaX *from the chromosome and replace it with a kanamycin resistance gene.*
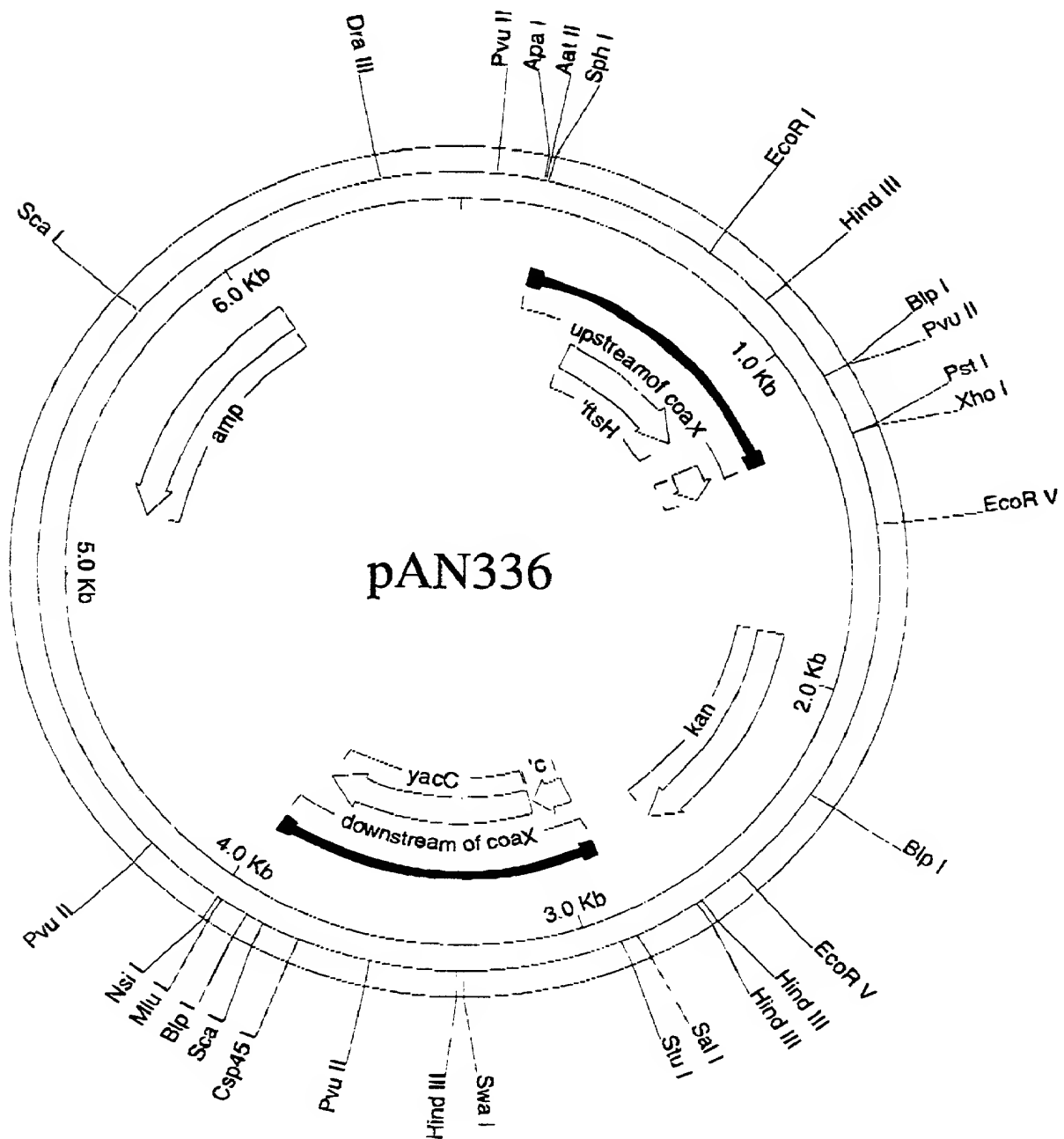
DECLARATION, PETITION AND POWER OF ATTORNEY FOR CONTINUATION-IN-PART PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

METHODS AND MICROORGANISMS FOR PRODUCTION OF PANTO-COMPOUNDS

the specification of which

    ☒   executed by me of even date herewith and about to be filed;

    ☐   was filed on _____ as Application Serial No. _____;
          and

    ☐   was amended on _____.
                             (if applicable)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

This application in part discloses and claims subject matter disclosed in my earlier filed application(s), as follows:

    ☒   Serial No.___09/400,494___ filed_____September 21, 1999___; as to which I claim priority benefit under Title 35, United States Code, §120.

    ☒   Serial No.___60/210,072___ filed_____June 7, 2000_____; as to which I claim priority benefit under Title 35, United States Code, §119(e).

    ☒   Serial No.___60/221,836___ filed_____July 28, 2000_____; as to which I claim priority benefit under Title 35, United States Code, §119(e).

    ☒   Serial No.___60/227,860___ filed_____August 24, 2000_____; as to which I claim priority benefit under Title 35, United States Code, §119(e).

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56, including all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application(s) and the national or PCT international filing date of the continuation-in-part application.

AS TO PARENT APPLICATION

As to the subject matter of this application which is common to said earlier application, I do not know and do not believe that the same was ever known or used in the United States of America before my or our invention thereof or patented or described in any printed publication in any country before my or our invention thereof, or more than one year prior to said earlier application, or in public use or on sale in the United States of America more than one year prior to said earlier application; that the common subject matter has not been patented or made the subject of an inventor's certificate issued before the date of said earlier application in any country foreign to the United States of America on an application filed by me or my legal representatives or assigns more than twelve months prior to said earlier application; and

As to applications for patents or inventor's certificate or PCT international application(s) designating at least one country other than the United States of America, on the common subject matter, filed in or designating any country foreign to the United States of America, prior to said earlier application by me or my legal representatives or assigns,

☒ no such applications have been filed.

☐ such applications have been filed as follows

EARLIEST FOREIGN APPLICATION(S), IF ANY, FILED WITHIN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO SAID EARLIER U.S. APPLICATION

Country	Application Number	Date of Filing (month,day,year)	Priority Claimed Under 35 USC 119
			☐ Yes ☐ No
			☐ Yes ☐ No
			☐ Yes ☐ No
			☐ Yes ☐ No
			☐ Yes ☐ No

ALL FOREIGN APPLICATION(S), IF ANY FILED MORE THAN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION

AS TO THIS APPLICATION

As to the subject matter of this application which is common to said earlier application, I do not know and do not believe that the same was ever known or used in the United States of America before my or our invention thereof or patented or described in any printed publication in any country before my or our invention thereof, or more than one year prior to said earlier application, or in public use or on sale in the United States of America more than one year prior to said earlier application; that the common subject matter has not been patented or made the subject of an inventor's certificate issued before the date of said earlier application in any country foreign to the United States of America on an application filed by me or my legal representatives or assigns more than twelve months prior to said earlier application; and

As to applications for patents or inventor's certificate or PCT international application(s) designating at least one country other than the United States of America, on the common subject matter, filed in or designating any country foreign to the United States of America, prior to said earlier application by me or my legal representatives or assigns,

    ☒   no such applications have been filed.

    ☐   such applications have been filed as follows

EARLIEST FOREIGN APPLICATION(S), IF ANY, FILED WITHIN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO SAID EARLIER U.S. APPLICATION

Country	Application Number	Date of Filing (month,day,year)	Priority Claimed Under 35 USC 119
			☐ Yes ☐ No
			☐ Yes ☐ No
			☐ Yes ☐ No
			☐ Yes ☐ No
			☐ Yes ☐ No

ALL FOREIGN APPLICATION(S), IF ANY FILED MORE THAN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION

CLAIM FOR BENEFIT OF U.S. PROVISIONAL APPLICATION(S)

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below.

60/210,072	June 7, 2000
(Application Serial No.)	(Filing Date)

60/221,836	July 28, 2000
(Application Serial No.)	(Filing Date)

60/227,860	August 24, 2000
(Application Serial No.)	(Filing Date)

CLAIM FOR BENEFIT OF U.S. PATENT APPLICATION(S)

I hereby claim the benefit under 35 U.S.C. §120 of any United States patent application(s) listed below.

09/400,494	September 21, 1999
(Application Serial No.)	(Filing Date)

(Application Serial No.)	(Filing Date)

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorneys and/or agents to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

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Giulio A. DeConti, Jr.	Reg. No. 31,503	William D. DeVaul	Reg. No. 42,483
Ann Lamport Hammitte	Reg. No. 34,858	David J. Rikkers	Reg. No. 43,882
Elizabeth A. Hanley	Reg. No. 33,505	Chi Suk Kim	Reg. No. 42,728
Amy E. Mandragouras	Reg. No. 36,207	Maria Laccotripe Zacharakis	Limited Recognition
Anthony A. Laurentano	Reg. No. 38,220		Under 37 C.F.R. § 10.9(b)
Jane E. Remillard	Reg. No. 38,872	Debra J. Milasincic	Reg. No. 46,931
Jeremiah Lynch	Reg. No. 17,425	David R. Burns	Reg. No. 46,590
Kevin J. Canning	Reg. No. 35,470	Sean D. Detweiler	Reg. No. 42,482
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Wherefore I petition that letters patent be granted to me for the invention or discovery described and claimed in the attached specification and claims, and hereby subscribe my name to said specification and claims and to the foregoing declaration, power of attorney, and this petition.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Citizenship United States of America	
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Citizenship United States of America	
Post Office Address (if different) Same as above	

SEQUENCE LISTING

<110> Yocum, R. et al.

<120> METHODS AND MICROORGANISMS FOR PRODUCTION OF
      PANTO-COMPOUNDS

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Gln Trp Ala Glu Leu Arg Lys Ser Val Pro Leu Lys Leu Thr Glu Gln
            20   25   30

Asp Leu Lys Pro Leu Leu Gly Phe Asn Glu Asp Leu Ser Leu Asp Glu
            35   40   45

Val Ser Thr Ile Tyr Leu Pro Leu Thr Arg Leu Ile Asn Tyr Tyr Ile
        50   55   60

Asp Glu Asn Leu His Arg Gln Thr Val Leu His Arg Phe Leu Gly Arg
 65   70   75   80

Asn Asn Ala Lys Thr Pro Tyr Ile Ile Ser Ile Ala Gly Ser Val Ala
                85   90   95

Val Gly Lys Ser Thr Ser Ala Arg Ile Leu Gln Ser Leu Leu Ser His
            100   105   110

Trp Pro Thr Glu Arg Lys Val Asp Leu Ile Thr Thr Asp Gly Phe Leu
            115   120   125

Tyr Pro Leu Asn Lys Leu Lys Gln Asp Asn Leu Leu Gln Lys Lys Gly
            130   135   140

Phe Pro Val Ser Tyr Asp Thr Pro Lys Leu Ile Arg Phe Leu Ala Asp
145   150   155   160

Val Lys Ser Gly Lys Ser Asn Val Thr Ala Pro Ile Tyr Ser His Leu
                165   170   175

Thr Tyr Asp Ile Ile Pro Asp Lys Phe Asp Val Val Asp Lys Pro Asp
            180   185   190

Ile Leu Ile Leu Glu Gly Leu Asn Val Leu Gln Thr Gly Asn Asn Lys
            195   200   205

Thr Asp Gln Thr Phe Val Ser Asp Phe Val Asp Phe Ser Ile Tyr Val
    210   215   220

Asp Ala Glu Glu Lys Leu Leu Lys Glu Trp Tyr Ile Lys Arg Phe Leu
225   230   235   240

Lys Phe Arg Glu Ser Ala Phe Asn Asp Pro Asn Ser Tyr Phe Lys His
            245   250   255

Tyr Ala Ser Leu Ser Lys Glu Glu Ala Ile Ala Thr Ala Ser Lys Ile
            260   265   270

Trp Asp Glu Ile Asn Gly Leu Asn Leu Asn Gln Asn Ile Leu Pro Thr
        275   280   285

Arg Glu Arg Ala Asn Leu Ile Leu Lys Lys Gly His Asn His Gln Val
    290   295   300

Glu Leu Ile Lys Leu Arg Lys
305   310

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Arg Asn Gln Trp Ala Ala Leu Arg Asp Ser Val Pro Met Thr Leu Ser
        20   25   30

Glu Asp Glu Ile Ala Arg Leu Lys Gly Ile Asn Glu Asp Leu Ser Leu
    35   40   45

Glu Glu Val Ala Glu Ile Tyr Leu Pro Leu Ser Arg Leu Leu Asn Phe
    50   55   60

Tyr Ile Ser Ser Asn Leu Arg Arg Gln Ala Val Leu Glu Gln Phe Leu
65   70   75   80

Gly Thr Asn Gly Gln Arg Ile Pro Tyr Ile Ile Ser Ile Ala Gly Ser
            85   90   95

Val Ala Val Gly Lys Ser Thr Thr Ala Arg Val Leu Gln Ala Leu Leu
        100   105   110

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Ser Arg Trp Pro Glu His Arg Arg Val Glu Leu Ile Thr Thr Asp Gly
        115             120             125

Phe Leu His Pro Asn Gln Val Leu Lys Glu Arg Gly Leu Met Lys Lys
        130             135             140

Lys Gly Phe Pro Glu Ser Tyr Asp Met His Arg Leu Val Lys Phe Val
145             150             155             160

Ser Asp Leu Lys Ser Gly Val Pro Asn Val Thr Ala Pro Val Tyr Ser
            165             170             175

His Leu Ile Tyr Asp Val Ile Pro Asp Gly Asp Lys Thr Val Val Gln
            180             185             190

Pro Asp Ile Leu Ile Leu Glu Gly Leu Asn Val Leu Gln Ser Gly Met
            195             200             205

Asp Tyr Pro His Asp Pro His His Val Phe Val Ser Asp Phe Val Asp
        210             215             220

Phe Ser Ile Tyr Val Asp Ala Pro Glu Asp Leu Leu Gln Thr Trp Tyr
225             230             235             240

Ile Asn Arg Phe Leu Lys Phe Arg Glu Gly Ala Phe Thr Asp Pro Asp
            245             250             255

Ser Tyr Phe His Asn Tyr Ala Lys Leu Thr Lys Glu Glu Ala Ile Lys
            260             265             270

Thr Ala Met Thr Leu Trp Lys Glu Ile Asn Trp Leu Asn Leu Lys Gln
            275             280             285

Asn Ile Leu Pro Thr Arg Glu Arg Ala Ser Leu Ile Leu Thr Lys Ser
        290             295             300

Ala Asn His Ala Val Glu Glu Val Arg Leu Arg Lys
305             310             315
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<213> Bacillus subtilis

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Met Lys Asn Lys Glu Leu Asn Leu His Thr Leu Tyr Thr Gln His Asn
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Arg Glu Ser Trp Ser Gly Phe Gly Gly His Leu Ser Ile Ala Val Ser
            20              25              30

Glu Glu Glu Ala Lys Ala Val Glu Gly Leu Asn Asp Tyr Leu Ser Val
        35              40              45

Glu Glu Val Glu Thr Ile Tyr Ile Pro Leu Val Arg Leu Leu His Leu
        50              55              60

His Val Lys Ser Ala Ala Glu Arg Asn Lys His Val Asn Val Phe Leu
 65              70              75              80
```

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Lys His Pro His Ser Ala Lys Ile Pro Phe Ile Ile Gly Ile Ala Gly
             85               90                  95

Ser Val Ala Val Gly Lys Ser Thr Thr Ala Arg Ile Leu Gln Lys Leu
             100              105              110

Leu Ser Arg Leu Pro Asp Arg Pro Lys Val Ser Leu Ile Thr Thr Asp
         115              120              125

Gly Phe Leu Phe Pro Thr Ala Glu Leu Lys Lys Lys Asn Met Met Ser
    130              135              140

Arg Lys Gly Phe Pro Glu Ser Tyr Asp Val Lys Ala Leu Leu Glu Phe
145              150              155              160

Leu Asn Asp Leu Lys Ser Gly Lys Asp Ser Val Lys Ala Pro Val Tyr
             165              170              175

Ser His Leu Thr Tyr Asp Arg Glu Glu Gly Val Phe Glu Val Val Glu
         180              185              190

Gln Ala Asp Ile Val Ile Ile Glu Gly Ile Asn Val Leu Gln Ser Pro
         195              200              205

Thr Leu Glu Asp Asp Arg Glu Asn Pro Arg Ile Phe Val Ser Asp Phe
    210              215              220

Phe Asp Phe Ser Ile Tyr Val Asp Ala Glu Glu Ser Arg Ile Phe Thr
225              230              235              240

Trp Tyr Leu Glu Arg Phe Arg Leu Leu Arg Glu Thr Ala Phe Gln Asn
             245              250              255

Pro Asp Ser Tyr Phe His Lys Phe Lys Asp Leu Ser Asp Gln Glu Ala
             260              265              270

Asp Glu Met Ala Ala Ser Ile Trp Glu Ser Val Asn Arg Pro Asn Leu
         275              280              285

Tyr Glu Asn Ile Leu Pro Thr Lys Phe Arg Ser Asp Leu Ile Leu Arg
    290              295              300

Lys Gly Asp Gly His Lys Val Glu Glu Val Leu Val Arg Arg Val
305              310              315
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<210> 4
<211> 312
<212> PRT
<213> Mycobacterium leprae

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Met Pro Arg Leu Ser Glu Pro Ser Pro Tyr Val Glu Phe Asp Arg Lys
  1               5               10                  15

Gln Trp Arg Ala Leu Arg Met Ser Thr Pro Leu Ala Leu Thr Glu Glu
             20               25               30

Glu Leu Ile Gly Leu Arg Gly Leu Gly Glu Gln Ile Asp Leu Leu Glu
         35               40               45
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Val Glu Glu Val Tyr Leu Pro Leu Ala Arg Leu Ile His Leu Gln Val
        50   55   60

Ala Ala Arg Gln Arg Leu Phe Ala Ala Thr Ala Glu Phe Leu Gly Glu
    65   70   75   80

Pro Gln Gln Asn Pro Gly Arg Pro Val Pro Phe Ile Ile Gly Val Ala
                85   90   95

Gly Ser Val Ala Val Gly Lys Ser Thr Thr Ala Arg Val Leu Gln Ala
            100   105   110

Leu Leu Ala Arg Trp Asp His His Thr Arg Val Asp Leu Val Thr Thr
        115   120   125

Asp Gly Phe Leu Tyr Pro Asn Ala Glu Leu Gly Arg Arg Asn Leu Met
    130   135   140

His Arg Lys Gly Phe Pro Glu Ser Tyr Asn Arg Arg Ala Leu Met Arg
145   150   155   160

Phe Val Thr Ser Val Lys Ser Gly Ala Asp Tyr Ala Cys Ala Pro Val
            165   170   175

Tyr Ser His Leu Arg Tyr Asp Thr Ile Pro Gly Ala Lys His Val Val
        180   185   190

Arg His Pro Asp Ile Leu Ile Leu Glu Gly Leu Asn Val Leu Gln Thr
        195   200   205

Gly Pro Thr Leu Met Val Ser Asp Leu Phe Asp Phe Ser Leu Tyr Val
    210   215   220

Asp Ala Arg Ile Gln Asp Ile Glu Gln Trp Tyr Val Ser Arg Phe Leu
225   230   235   240

Ala Met Arg Gly Thr Ala Phe Ala Asp Pro Glu Ser His Phe His His
            245   250   255

Tyr Ser Ala Leu Thr Asp Ser Lys Ala Ile Ile Ala Ala Arg Glu Ile
            260   265   270

Trp Arg Ser Ile Asn Arg Pro Asn Leu Val Glu Asn Ile Leu Pro Thr
        275   280   285

Arg Pro Arg Ala Thr Leu Val Leu Arg Lys Asp Ala Asp His Ser Ile
    290   295   300

Asn Arg Leu Arg Leu Arg Lys Leu
305   310

<210> 5
<211> 312
<212> PRT
<213> Mycobacterium tuberculosis

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Met Ser Arg Leu Ser Glu Pro Ser Pro Tyr Val Glu Phe Asp Arg Arg
  1   5   10   15

Gln Trp Arg Ala Leu Arg Met Ser Thr Pro Leu Ala Leu Thr Glu Glu
            20   25   30

Glu Leu Val Gly Leu Arg Gly Leu Gly Glu Gln Ile Asp Leu Leu Glu
        35   40   45

Val Glu Glu Val Tyr Leu Pro Leu Ala Arg Leu Ile His Leu Gln Val
    50   55   60

Ala Ala Arg Gln Arg Leu Phe Ala Ala Thr Ala Glu Phe Leu Gly Glu
65   70   75   80

Pro Gln Gln Asn Pro Asp Arg Pro Val Pro Phe Ile Ile Gly Val Ala
            85   90   95

Gly Ser Val Ala Val Gly Lys Ser Thr Thr Ala Arg Val Leu Gln Ala
        100   105   110

Leu Leu Ala Arg Trp Asp His His Pro Arg Val Asp Leu Val Thr Thr
        115   120   125

Asp Gly Phe Leu Tyr Pro Asn Ala Glu Leu Gln Arg Arg Asn Leu Met
    130   135   140

His Arg Lys Gly Phe Pro Glu Ser Tyr Asn Arg Arg Ala Leu Met Arg
145   150   155   160

Phe Val Thr Ser Val Lys Ser Gly Ser Asp Tyr Ala Cys Ala Pro Val
            165   170   175

Tyr Ser His Leu His Tyr Asp Ile Ile Pro Gly Ala Glu Gln Val Val
        180   185   190

Arg His Pro Asp Ile Leu Ile Leu Glu Gly Leu Asn Val Leu Gln Thr
        195   200   205

Gly Pro Thr Leu Met Val Ser Asp Leu Phe Asp Phe Ser Leu Tyr Val
    210   215   220

Asp Ala Arg Ile Glu Asp Ile Glu Gln Trp Tyr Val Ser Arg Phe Leu
225   230   235   240

Ala Met Arg Thr Thr Ala Phe Ala Asp Pro Glu Ser His Phe His His
            245   250   255

Tyr Ala Ala Phe Ser Asp Ser Gln Ala Val Val Ala Ala Arg Glu Ile
        260   265   270

Trp Arg Thr Ile Asn Arg Pro Asn Leu Val Glu Asn Ile Leu Pro Thr
        275   280   285

Arg Pro Arg Ala Thr Leu Val Leu Arg Lys Asp Ala Asp His Ser Ile
    290   295   300

Asn Arg Leu Arg Leu Arg Lys Leu
305   310

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<212> PRT

<213> Streptomyces coelicolor

<400> 6

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Pro Glu Ala Thr Pro Tyr Val Asp Leu Thr Arg Pro Glu Trp Ser Ala
                20                  25                  30

Leu Arg Asp Lys Thr Pro Leu Pro Leu Thr Ala Glu Glu Val Glu Lys
            35                  40                  45

Leu Arg Gly Leu Gly Asp Val Ile Asp Leu Asp Glu Val Arg Asp Ile
        50                  55                  60

Tyr Leu Pro Leu Ser Arg Leu Leu Asn Leu Tyr Val Gly Ala Thr Asp
65                  70                  75                  80

Gly Leu Arg Gly Ala Leu Asn Thr Phe Leu Gly Glu Gln Gly Ser Gln
                85                  90                  95

Ser Gly Thr Pro Phe Val Ile Gly Val Ala Gly Ser Val Ala Val Gly
            100                 105                 110

Lys Ser Thr Val Ala Arg Leu Leu Gln Ala Leu Leu Ser Arg Trp Pro
        115                 120                 125

Glu His Pro Arg Val Glu Leu Val Thr Thr Asp Gly Phe Leu Leu Pro
    130                 135                 140

Thr Arg Glu Leu Glu Ala Arg Gly Leu Met Ser Arg Lys Gly Phe Pro
145                 150                 155                 160

Glu Ser Tyr Asp Arg Arg Ala Leu Thr Arg Phe Val Ala Asp Ile Lys
                165                 170                 175

Ala Gly Lys Ala Glu Val Thr Ala Pro Val Tyr Ser His Leu Ile Tyr
            180                 185                 190

Asp Ile Val Pro Asp Gln Arg Leu Val Val Arg Arg Pro Asp Ile Leu
            195                 200                 205

Ile Val Glu Gly Leu Asn Val Leu Gln Pro Ala Leu Pro Gly Lys Asp
    210                 215                 220

Gly Arg Thr Arg Val Gly Leu Ala Asp Tyr Phe Asp Phe Ser Val Tyr
225                 230                 235                 240

Val Asp Ala Arg Thr Glu Asp Ile Glu Arg Trp Tyr Leu Asn Arg Phe
                245                 250                 255

Arg Lys Leu Arg Ala Thr Ala Phe Gln Asn Pro Ser Ser Tyr Phe Arg
            260                 265                 270

Lys Tyr Thr Gln Val Ser Glu Glu Glu Ala Leu Asp Tyr Ala Arg Thr
        275                 280                 285

Thr Trp Arg Thr Ile Asn Lys Pro Asn Leu Val Glu Asn Val Ala Pro
        290                 295                 300

Thr Arg Gly Arg Ala Thr Leu Val Leu Arg Lys Gly Pro Asp His Lys
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Val Gln Arg Leu Ser Leu Arg Lys Leu
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<213> Streptomyces coelicolor

<400> 7
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Phe Asp Gly Glu Asp Ile Val Glu His Trp Arg Ile Ser Thr Asp Ser
            20   25   30

Arg Arg Thr Ala Asp Glu Leu Ala Val Leu Leu Gln Gly Leu Met Gly
          35   40   45

Met His Pro Leu Leu Gly Asp Glu Leu Gly Asp Gly Ile Asp Gly Ile
        50   55   60

Ala Ile Cys Ala Thr Val Pro Ser Val Leu His Glu Leu Arg Glu Val
65   70   75   80

Thr Arg Arg Tyr Tyr Gly Asp Val Pro Ala Val Leu Val Glu Pro Gly
                85   90   95

Val Lys Thr Gly Val Pro Ile Leu Thr Asp His Pro Lys Glu Val Gly
              100   105   110

Ala Asp Arg Ile Ile Asn Ala Val Ala Ala Val Glu Leu Tyr Gly Gly
            115   120   125

Pro Ala Ile Val Val Asp Phe Gly Thr Ala Thr Thr Phe Asp Ala Val
        130   135   140

Ser Ala Arg Gly Glu Tyr Ile Gly Gly Val Ile Ala Pro Gly Ile Glu
145   150   155   160

Ile Ser Val Glu Ala Leu Gly Val Lys Gly Ala Gln Leu Arg Lys Ile
                165   170   175

Glu Val Ala Arg Pro Arg Ser Val Ile Gly Lys Asn Thr Val Glu Ala
            180   185   190

Met Gln Ser Gly Ile Val Tyr Gly Phe Ala Gly Gln Val Asp Gly Val
          195   200   205

Val Asn Arg Met Ala Arg Glu Leu Ala Asp Asp Pro Asp Asp Val Thr
        210   215   220

Val Ile Ala Thr Gly Gly Leu Ala Pro Met Val Leu Gly Glu Ser Ser
225   230   235   240

Val Ile Asp Glu His Glu Pro Trp Leu Thr Leu Met Gly Leu Arg Leu
                245   250   255

Val Tyr Glu Arg Asn Val Ser Arg Met

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            260                      265


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<211> 272
<212> PRT
<213> Mycobacterium tuberculosis

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             20              25                  30

Arg Thr Glu Ser Glu Val Thr Ala Asp Glu Leu Ala Leu Thr Ile Asp
         35                  40                  45

Gly Leu Ile Gly Glu Asp Ser Glu Arg Leu Thr Gly Thr Ala Ala Leu
     50                  55                  60

Ser Thr Val Pro Ser Val Leu His Glu Val Arg Ile Met Leu Asp Gln
65                  70                  75                  80

Tyr Trp Pro Ser Val Pro His Val Leu Ile Glu Pro Gly Val Arg Thr
                 85                  90                  95

Gly Ile Pro Leu Leu Val Asp Asn Pro Lys Glu Val Gly Ala Asp Arg
             100                 105                 110

Ile Val Asn Cys Leu Ala Ala Tyr Asp Arg Phe Arg Lys Ala Ala Ile
             115                 120                 125

Val Val Asp Phe Gly Ser Ser Ile Cys Val Asp Val Val Ser Ala Lys
     130                 135                 140

Gly Glu Phe Leu Gly Gly Ala Ile Ala Pro Gly Val Gln Val Ser Ser
145                 150                 155                 160

Asp Ala Ala Ala Ala Arg Ser Ala Ala Leu Arg Arg Val Glu Leu Ala
                 165                 170                 175

Arg Pro Arg Ser Val Val Gly Lys Asn Thr Val Glu Cys Met Gln Ala
             180                 185                 190

Gly Ala Val Phe Gly Phe Ala Gly Leu Val Asp Gly Leu Val Gly Arg
             195                 200                 205

Ile Arg Glu Asp Val Ser Gly Phe Ser Val Asp His Asp Val Ala Ile
     210                 215                 220

Val Ala Thr Gly His Thr Ala Pro Leu Leu Leu Pro Glu Leu His Thr
225                 230                 235                 240

Val Asp His Tyr Asp Gln His Leu Thr Leu Gln Gly Leu Arg Leu Val
                 245                 250                 255

Phe Glu Arg Asn Leu Glu Val Gln Arg Gly Arg Leu Lys Thr Ala Arg
             260                 265                 270
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<211> 258
<212> PRT
<213> Bacillus subtilis

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 1               5                  10                  15


Tyr His Asp Gly Lys Leu Glu Tyr His Trp Arg Ile Glu Thr Ser Arg
            20                  25                  30


His Lys Thr Glu Asp Glu Phe Gly Met Ile Leu Arg Ser Leu Phe Asp
        35                  40                  45


His Ser Gly Leu Met Phe Glu Gln Ile Asp Gly Ile Ile Ile Ser Ser
    50                  55                  60


Val Val Pro Pro Ile Met Phe Ala Leu Glu Arg Met Cys Thr Lys Tyr
65                  70                  75                  80


Phe His Ile Glu Pro Gln Ile Val Gly Pro Gly Met Lys Thr Gly Leu
                85                  90                  95


Asn Ile Lys Tyr Asp Asn Pro Lys Glu Val Gly Ala Asp Arg Ile Val
            100                 105                 110


Asn Ala Val Ala Ala Ile His Leu Tyr Gly Asn Pro Leu Ile Val Val
        115                 120                 125


Asp Phe Gly Thr Ala Thr Thr Tyr Cys Tyr Ile Asp Glu Asn Lys Gln
    130                 135                 140


Tyr Met Gly Gly Ala Ile Ala Pro Gly Ile Thr Ile Ser Thr Glu Ala
145                 150                 155                 160


Leu Tyr Ser Arg Ala Ala Lys Leu Pro Arg Ile Glu Ile Thr Arg Pro
                165                 170                 175


Asp Asn Ile Ile Gly Lys Asn Thr Val Ser Ala Met Gln Ser Gly Ile
            180                 185                 190


Leu Phe Gly Tyr Val Gly Gln Val Glu Gly Ile Val Lys Arg Met Lys
            195                 200                 205


Trp Gln Ala Lys Gln Asp Leu Lys Val Ile Ala Thr Gly Gly Leu Ala
    210                 215                 220


Pro Leu Ile Ala Asn Glu Ser Asp Cys Ile Asp Ile Val Asp Pro Phe
225                 230                 235                 240


Leu Thr Leu Lys Gly Leu Glu Leu Ile Tyr Glu Arg Asn Arg Val Gly
                245                 250                 255


Ser Val
```

<210> 10
<211> 262
<212> PRT
<213> Deinococcus radiopugnans

<400> 10

```
Met Pro Ala Phe Pro Leu Leu Ala Val Asp Ile Gly Asn Thr Thr Thr
 1               5                  10                  15

Val Leu Gly Leu Ala Asp Ala Ser Gly Ala Leu Thr His Thr Trp Arg
        20                  25                  30

Ile Arg Thr Asn Arg Glu Met Leu Pro Asp Asp Leu Ala Leu Gln Leu
            35                  40                  45

His Gly Leu Phe Thr Leu Ala Gly Ala Pro Ile Pro Arg Ala Ala Val
        50                  55                  60

Leu Ser Ser Val Ala Pro Pro Val Gly Glu Asn Tyr Ala Leu Ala Leu
65                  70                  75                  80

Lys Arg His Phe Met Ile Asp Ala Phe Ala Val Ser Ala Glu Asn Leu
                85                  90                  95

Pro Asp Val Thr Val Glu Leu Asp Thr Pro Gly Ser Val Gly Ala Asp
            100                 105                 110

Arg Leu Cys Asn Leu Phe Gly Ala Glu Lys Tyr Leu Gly Gly Leu Asp
        115                 120                 125

Tyr Ala Val Val Val Asp Phe Gly Thr Ser Thr Asn Phe Asp Val Val
        130                 135                 140

Gly Arg Gly Arg Arg Phe Leu Gly Gly Ile Leu Ala Thr Gly Ala Gln
145                 150                 155                 160

Val Ser Ala Asp Ala Leu Phe Ala Arg Ala Ala Lys Leu Pro Arg Ile
                165                 170                 175

Thr Leu Gln Ala Pro Glu Thr Ala Ile Gly Lys Asn Thr Val His Ala
            180                 185                 190

Leu Gln Ser Gly Leu Val Phe Gly Tyr Ala Glu Met Val Asp Gly Leu
        195                 200                 205

Leu Arg Arg Ile Arg Ala Glu Leu Pro Gly Glu Ala Val Ala Val Ala
        210                 215                 220

Thr Gly Gly Phe Ser Arg Thr Val Gln Gly Ile Cys Gln Glu Ile Asp
225                 230                 235                 240

Tyr Tyr Asp Glu Thr Leu Thr Leu Arg Gly Leu Val Glu Leu Trp Ala
                245                 250                 255

Ser Arg Ser Glu Val Arg
            260
```

<210> 11
<211> 212
<212> PRT

<213> Desulfovibrio vulgaris

<400> 11
Met Thr Gln His Phe Leu Leu Phe Asp Ile Gly Asn Thr Asn Val Lys
1   5   10   15

Ile Gly Ile Ala Val Glu Thr Ala Val Leu Thr Ser Tyr Val Leu Pro
                20   25   30

Thr Asp Pro Gly Gln Thr Thr Asp Ser Ile Gly Leu Arg Leu Leu Glu
            35   40   45

Val Leu Arg His Ala Gly Leu Gly Pro Ala Asp Val Gly Ala Cys Val
        50   55   60

Ala Ser Ser Val Val Pro Gly Val Asn Pro Leu Ile Arg Arg Ala Cys
65   70   75   80

Glu Arg Tyr Leu Tyr Arg Lys Leu Leu Phe Ala Pro Gly Asp Ile Ala
            85   90   95

Ile Pro Leu Asp Asn Arg Tyr Glu Arg Pro Ala Glu Val Gly Ala Asp
            100   105   110

Arg Leu Val Ala Ala Tyr Ala Ala Arg Arg Leu Tyr Pro Gly Pro Arg
        115   120   125

Ser Leu Val Ser Val Asp Phe Gly Thr Ala Thr Thr Phe Asp Cys Val
    130   135   140

Glu Gly Gly Ala Tyr Leu Gly Gly Leu Ile Cys Pro Gly Val Leu Ser
145   150   155   160

Ser Ala Gly Ala Leu Ser Ser Arg Thr Ala Lys Leu Pro Arg Ile Ser
            165   170   175

Leu Glu Val Glu Glu Asp Ser Pro Val Ile Gly Arg Ser Thr Thr Thr
        180   185   190

Ser Leu Asn His Gly Phe Ile Phe Gly Phe Ala Ala Met Thr Glu Gly
        195   200   205

Val Leu Ala Ala
    210

<210> 12
<211> 246
<212> PRT
<213> Thermotoga maritima

<400> 12
Met Tyr Leu Leu Val Asp Val Gly Asn Thr His Ser Val Phe Ser Ile
1   5   10   15

Thr Glu Asp Gly Lys Thr Phe Arg Arg Trp Arg Leu Ser Thr Gly Val
                20   25   30

Phe Gln Thr Glu Asp Glu Leu Phe Ser His Leu His Pro Leu Leu Gly
            35   40   45

Asp Ala Met Arg Glu Ile Lys Gly Ile Gly Val Ala Ser Val Val Pro
   50   55   60

Thr Gln Asn Thr Val Ile Glu Arg Phe Ser Gln Lys Tyr Phe His Ile
 65   70   75   80

Ser Pro Ile Trp Val Lys Ala Lys Asn Gly Cys Val Lys Trp Asn Val
         85   90   95

Lys Asn Pro Ser Glu Val Gly Ala Asp Arg Val Ala Asn Val Val Ala
         100   105   110

Phe Val Lys Glu Tyr Gly Lys Asn Gly Ile Ile Ile Asp Met Gly Thr
       115   120   125

Ala Thr Thr Val Asp Leu Val Val Asn Gly Ser Tyr Glu Gly Gly Ala
   130   135   140

Ile Leu Pro Gly Phe Phe Met Met Val His Ser Leu Phe Arg Gly Thr
145   150   155   160

Ala Lys Leu Pro Leu Val Glu Val Lys Pro Ala Asp Phe Val Val Gly
         165   170   175

Lys Asp Thr Glu Glu Asn Ile Arg Leu Gly Val Val Asn Gly Ser Val
       180   185   190

Tyr Ala Leu Glu Gly Ile Ile Gly Arg Ile Lys Glu Val Tyr Gly Asp
       195   200   205

Leu Pro Val Val Leu Thr Gly Gly Gln Ser Lys Ile Val Lys Asp Met
   210   215   220

Ile Lys His Glu Ile Phe Asp Glu Asp Leu Thr Ile Lys Gly Val Tyr
225   230   235   240

His Phe Cys Phe Gly Asp
         245

<210> 13
<211> 273
<212> PRT
<213> Treponema pallidum

<400> 13
Met Leu Leu Ile Asp Val Gly Asn Ser His Val Val Phe Gly Ile Gln
  1   5   10   15

Gly Glu Asn Gly Gly Arg Val Cys Val Arg Glu Leu Phe Arg Leu Ala
        20   25   30

Pro Asp Ala Arg Lys Thr Gln Asp Glu Tyr Ser Leu Leu Ile His Ala
      35   40   45

Leu Cys Glu Arg Ala Gly Val Gly Arg Ala Ser Leu Arg Asp Ala Phe
    50   55   60

Ile Ser Ser Val Val Pro Val Leu Thr Lys Thr Ile Ala Asp Ala Val
 65   70   75   80

Ala Gln Ile Ser Gly Val Gln Pro Val Val Phe Gly Pro Trp Ala Tyr
              85   90   95

Glu His Leu Pro Val Arg Ile Pro Glu Pro Val Arg Ala Glu Ile Gly
              100   105   110

Thr Asp Leu Val Ala Asn Ala Val Ala Ala Tyr Val His Phe Arg Ser
          115   120   125

Ala Cys Val Val Val Asp Cys Gly Thr Ala Leu Thr Phe Thr Ala Val
    130   135   140

Asp Gly Thr Gly Leu Ile Gln Gly Val Ala Ile Ala Pro Gly Leu Arg
145   150   155   160

Thr Ala Val Gln Ser Leu His Thr Gly Thr Ala Gln Leu Pro Leu Val
              165   170   -   175

Pro Leu Ala Leu Pro Asp Ser Val Leu Gly Lys Asp Thr Thr His Ala
          180   185   190

Val Gln Ala Gly Val Val Arg Gly Thr Leu Phe Val Ile Arg Ala Met
          195   200   205

Ile Ala Gln Cys Gln Lys Glu Leu Gly Cys Arg Cys Ala Ala Val Ile
    210   215   220

Thr Gly Gly Leu Ser Arg Leu Phe Ser Ser Glu Val Asp Phe Pro Pro
225   230   235   240

Ile Asp Ala Gln Leu Thr Leu Ser Gly Leu Ala His Ile Ala Arg Leu
              245   250   255

Val Pro Thr Ser Leu Leu Pro Pro Ala Thr Val Ser Gly Ser Ser Gly
              260   265   270

Asn

<210> 14
<211> 262
<212> PRT
<213> Borrelia burgdorferi

<400> 14
Met Asn Lys Pro Leu Leu Ser Glu Leu Ile Ile Asp Ile Gly Asn Thr
  1   5   10   15

Ser Ile Ala Phe Ala Leu Phe Lys Asp Asn Gln Val Asn Leu Phe Ile
              20   25   30

Lys Met Lys Thr Asn Leu Met Leu Arg Tyr Asp Glu Val Tyr Ser Phe
          35   40   45

Phe Glu Glu Asn Phe Asp Phe Asn Val Asn Lys Val Phe Ile Ser Ser
      50   55   60

Val Val Pro Ile Leu Asn Glu Thr Phe Lys Asn Val Ile Phe Ser Phe
  65   70   75   80

```
Phe Lys Ile Lys Pro Leu Phe Ile Gly Phe Asp Leu Asn Tyr Asp Leu
             85              90                  95

Thr Phe Asn Pro Tyr Lys Ser Asp Lys Phe Leu Leu Gly Ser Asp Val
            100             105             110

Phe Ala Asn Leu Val Ala Ala Ile Glu Asn Tyr Ser Phe Glu Asn Val
            115             120             125

Leu Val Val Asp Leu Gly Thr Ala Cys Thr Ile Phe Ala Val Ser Arg
        130             135             140

Gln Asp Gly Ile Leu Gly Gly Ile Ile Asn Ser Gly Pro Leu Ile Asn
145             150             155             160

Phe Asn Ser Leu Leu Asp Asn Ala Tyr Leu Ile Lys Lys Phe Pro Ile
            165             170             175

Ser Thr Pro Asn Asn Leu Leu Glu Arg Thr Thr Ser Gly Ser Val Asn
            180             185             190

Ser Gly Leu Phe Tyr Gln Tyr Lys Tyr Leu Ile Glu Gly Val Tyr Arg
        195             200             205

Asp Ile Lys Gln Met Tyr Lys Lys Lys Phe Asn Leu Ile Ile Thr Gly
        210             215             220

Gly Asn Ala Asp Leu Ile Leu Ser Leu Ile Glu Ile Glu Phe Ile Phe
225             230             235             240

Asn Ile His Leu Thr Val Glu Gly Val Arg Ile Leu Gly Asn Ser Ile
            245             250             255

Asp Phe Lys Phe Val Asn
            260
```

```
<210> 15
<211> 229
<212> PRT
<213> Aquifex aeolicus
```

```
<400> 15
Met Arg Phe Leu Thr Val Asp Val Gly Asn Ser Ser Val Asp Ile Ala
 1           5               10              15

Leu Trp Glu Gly Lys Lys Val Lys Asp Phe Leu Lys Leu Ser His Glu
            20              25              30

Glu Phe Leu Lys Glu Glu Phe Pro Lys Leu Lys Ala Leu Gly Ile Ser
        35              40              45

Val Lys Gln Ser Phe Ser Glu Lys Val Arg Gly Lys Ile Pro Lys Ile
        50              55              60

Lys Phe Leu Lys Lys Glu Asn Phe Pro Ile Gln Val Asp Tyr Lys Thr
65              70              75              80

Pro Glu Thr Leu Gly Thr Asp Arg Val Ala Leu Ala Tyr Ser Ala Lys
            85              90              95
```

```
Lys Phe Tyr Gly Lys Asn Val Val Val Ile Ser Ala Gly Thr Ala Leu
            100             105             110

Val Ile Asp Leu Val Leu Glu Gly Lys Phe Lys Gly Gly Phe Ile Thr
            115             120             125

Leu Gly Leu Gly Lys Lys Leu Lys Ile Leu Ser Asp Leu Ala Glu Gly
        130             135             140

Ile Pro Glu Phe Phe Pro Glu Glu Val Glu Ile Phe Leu Gly Arg Ser
145             150             155             160

Thr Arg Glu Cys Val Leu Gly Gly Ala Tyr Arg Glu Ser Thr Glu Phe
                165             170             175

Ile Lys Ser Thr Leu Lys Leu Trp Arg Lys Val Phe Lys Arg Lys Phe
            180             185             190

Lys Val Val Ile Thr Gly Gly Glu Gly Lys Tyr Phe Ser Lys Phe Gly
        195             200             205

Ile Tyr Asp Pro Leu Leu Val His Arg Gly Met Arg Asn Leu Leu Tyr
    210             215             220

Leu Tyr His Arg Ile
225


<210> 16
<211> 257
<212> PRT
<213> Synechocystis sp.

<400> 16
Met Glu Thr Ser Lys Pro Gly Cys Gly Leu Ala Leu Asp Asn Asp Lys
1           5               10              15

Gln Lys Pro Trp Leu Gly Leu Met Ile Gly Asn Ser Arg Leu His Trp
            20              25              30

Ala Tyr Cys Ser Gly Asn Ala Pro Leu Gln Thr Trp Val Thr Asp Tyr
        35              40              45

Asn Pro Lys Ser Ala Gln Leu Pro Val Leu Leu Gly Lys Val Pro Leu
    50              55              60

Met Leu Ala Ser Val Val Pro Glu Gln Thr Glu Val Trp Arg Val Tyr
65              70              75              80

Gln Pro Lys Ile Leu Thr Leu Lys Asn Leu Pro Leu Val Asn Leu Tyr
                85              90              95

Pro Ser Phe Gly Ile Asp Arg Ala Leu Ala Gly Leu Gly Thr Gly Leu
            100             105             110

Thr Tyr Gly Phe Pro Cys Leu Val Val Asp Gly Gly Thr Ala Leu Thr
        115             120             125

Ile Thr Gly Phe Asp Gln Asp Lys Lys Leu Val Gly Gly Ala Ile Leu
    130             135             140
```

```
Pro Gly Leu Gly Leu Gln Leu Ala Thr Leu Gly Asp Arg Leu Ala Ala
145                 150             155                 160

Leu Pro Lys Leu Glu Met Asp Gln Leu Thr Glu Leu Pro Asp Arg Trp
                165             170                 175

Ala Leu Asp Thr Pro Ser Ala Ile Phe Ser Gly Val Val Tyr Gly Val
            180             185                 190

Leu Gly Ala Leu Gln Ser Tyr Leu Gln Asp Trp Gln Lys Leu Phe Pro
            195             200                 205

Gly Ala Ala Met Val Ile Thr Gly Gly Asp Gly Lys Ile Leu His Gly
        210             215                 220

Phe Leu Lys Glu His Ser Pro Asn Leu Ser Val Ala Trp Asp Asp Asn
225                 230             235                 240

Leu Ile Phe Leu Gly Met Ala Ala Ile His His Gly Asp Arg Pro Ile
                245             250                 255

Cys
```

```
<210> 17
<211> 223
<212> PRT
<213> Helicobacter pylori
```

```
<400> 17
Met Pro Ala Arg Gln Ser Phe Thr Asp Leu Lys Asn Leu Val Leu Cys
  1             5               10                  15

Asp Ile Gly Asn Thr Arg Ile His Phe Ala Gln Asn Tyr Gln Leu Phe
                20              25                  30

Ser Ser Ala Lys Glu Asp Leu Lys Arg Leu Gly Ile Gln Lys Glu Ile
            35              40                  45

Phe Tyr Ile Ser Val Asn Glu Glu Asn Glu Lys Ala Leu Leu Asn Cys
        50              55                  60

Tyr Pro Asn Ala Lys Asn Ile Ala Gly Phe Phe His Leu Glu Thr Asp
 65                 70              75                  80

Tyr Val Gly Leu Gly Ile Asp Arg Gln Met Ala Cys Leu Ala Val Asn
                85              90                  95

Asn Gly Val Val Val Asp Ala Gly Ser Ala Ile Thr Ile Asp Leu Ile
            100             105                 110

Lys Glu Gly Lys His Leu Gly Gly Cys Ile Leu Pro Gly Leu Ala Gln
            115             120                 125

Tyr Ile His Ala Tyr Lys Lys Ser Ala Lys Ile Leu Glu Gln Pro Phe
        130             135                 140

Lys Ala Leu Asp Ser Leu Glu Val Leu Pro Lys Ser Thr Arg Asp Ala
145                 150             155                 160
```

```
Val Asn Tyr Gly Met Val Leu Ser Val Ile Ala Cys Ile Gln His Leu
                165             170             175

Ala Lys Asn Gln Lys Ile Tyr Leu Cys Gly Gly Asp Ala Lys Tyr Leu
                180             185             190

Ser Ala Phe Leu Pro His Ser Val Cys Lys Glu Arg Leu Val Phe Asp
            195             200             205

Gly Met Glu Ile Ala Leu Lys Lys Ala Gly Ile Leu Glu Cys Lys
    210             215             220
```

<210> 18
<211> 267
<212> PRT
<213> Bordetella pertussis

<400> 18
```
Met Ile Ile Leu Ile Asp Ser Gly Asn Ser Arg Leu Lys Val Gly Trp
  1           5               10              15

Phe Asp Pro Asp Ala Pro Gln Ala Ala Arg Glu Pro Ala Pro Val Ala
                20              25              30

Phe Asp Asn Leu Asp Leu Asp Ala Leu Gly Arg Trp Leu Ala Thr Leu
            35              40              45

Pro Arg Arg Pro Gln Arg Ala Leu Gly Val Asn Val Ala Gly Leu Ala
        50              55              60

Arg Gly Glu Ala Ile Ala Ala Thr Leu Arg Ala Gly Gly Cys Asp Ile
65              70              75              80

Arg Trp Leu Arg Ala Gln Pro Leu Ala Met Gly Leu Arg Asn Gly Tyr
            85              90              95

Arg Asn Pro Asp Gln Leu Gly Ala Asp Arg Trp Ala Cys Met Val Gly
            100             105             110

Val Leu Ala Arg Gln Pro Ser Val His Pro Pro Leu Leu Val Ala Ser
        115             120             125

Phe Gly Thr Ala Thr Thr Leu Asp Thr Ile Gly Pro Asp Asn Val Phe
    130             135             140

Pro Gly Gly Leu Ile Leu Pro Gly Pro Ala Met Met Arg Gly Ala Leu
145             150             155             160

Ala Tyr Gly Thr Ala His Leu Pro Leu Ala Asp Gly Leu Val Ala Asp
                165             170             175

Tyr Pro Ile Asp Thr His Gln Ala Ile Ala Ser Gly Ile Ala Ala Ala
            180             185             190

Gln Ala Gly Ala Ile Val Arg Gln Trp Leu Ala Gly Arg Gln Arg Tyr
        195             200             205

Gly Gln Ala Pro Glu Ile Tyr Val Ala Gly Gly Gly Trp Pro Glu Val
    210             215             220
```

```
Arg Gln Glu Ala Glu Arg Leu Leu Ala Val Thr Gly Ala Ala Phe Gly
225                 230                 235                 240

Ala Thr Pro Gln Pro Thr Tyr Leu Asp Ser Pro Val Leu Asp Gly Leu
                245                 250                 255

Ala Ala Leu Ala Ala Gln Gly Ala Pro Thr Ala
                260                 265
```

<210> 19
<211> 777
<212> DNA
<213> Bacillus subtilis

<220>
<221> CDS
<222> (1)..(774)

<400> 19

```
ttg tta ctg gtt atc gat gtg ggg aac acc aat act gta ctt ggt gta   48
Leu Leu Leu Val Ile Asp Val Gly Asn Thr Asn Thr Val Leu Gly Val
 1               5                  10                  15

tat cat gat gga aaa tta gaa tat cac tgg cgt ata gaa aca agc agg   96
Tyr His Asp Gly Lys Leu Glu Tyr His Trp Arg Ile Glu Thr Ser Arg
                20                  25                  30

cat aaa aca gaa gat gag ttt ggg atg att ttg cgc tcc tta ttt gat  144
His Lys Thr Glu Asp Glu Phe Gly Met Ile Leu Arg Ser Leu Phe Asp
                35                  40                  45

cac tcc ggg ctt atg ttt gaa cag ata gat ggc att att att tcg tca  192
His Ser Gly Leu Met Phe Glu Gln Ile Asp Gly Ile Ile Ile Ser Ser
        50                  55                  60

gta gtg ccg cca atc atg ttt gcg tta gaa aga atg tgc aca aaa tac  240
Val Val Pro Pro Ile Met Phe Ala Leu Glu Arg Met Cys Thr Lys Tyr
65                  70                  75                  80

ttt cat atc gag cct caa att gtt ggt cca ggt atg aaa acc ggt tta  288
Phe His Ile Glu Pro Gln Ile Val Gly Pro Gly Met Lys Thr Gly Leu
                85                  90                  95

aat ata aaa tat gac aat ccg aaa gaa gta ggg gca gac aga atc gta  336
Asn Ile Lys Tyr Asp Asn Pro Lys Glu Val Gly Ala Asp Arg Ile Val
                100                 105                 110

aat gct gtc gct gcg ata cac ttg tac ggc aat cca tta att gtt gtc  384
Asn Ala Val Ala Ala Ile His Leu Tyr Gly Asn Pro Leu Ile Val Val
                115                 120                 125

gat ttc gga acc gcc aca acg tac tgc tat att gat gaa aac aaa caa  432
Asp Phe Gly Thr Ala Thr Thr Tyr Cys Tyr Ile Asp Glu Asn Lys Gln
        130                 135                 140

tac atg ggc ggg gcg att gcc cct ggg att aca att tcg aca gag gcg  480
Tyr Met Gly Gly Ala Ile Ala Pro Gly Ile Thr Ile Ser Thr Glu Ala
145                 150                 155                 160

ctt tac tcg cgt gca gca aag ctt cct cgt atc gaa atc acc cgg ccc  528
```

```
Leu Tyr Ser Arg Ala Ala Lys Leu Pro Arg Ile Glu Ile Thr Arg Pro
            165             170             175

gac aat att atc gga aaa aac act gtt agc gcg atg caa tct gga att    576
Asp Asn Ile Ile Gly Lys Asn Thr Val Ser Ala Met Gln Ser Gly Ile
            180             185             190

tta ttt ggc tat gtc ggc caa gtg gaa gga atc gtt aag cga atg aaa    624
Leu Phe Gly Tyr Val Gly Gln Val Glu Gly Ile Val Lys Arg Met Lys
            195             200             205

tgg cag gca aaa cag gac ctc aag gtc att gcg aca gga ggc ctg gcg    672
Trp Gln Ala Lys Gln Asp Leu Lys Val Ile Ala Thr Gly Gly Leu Ala
        210             215             220

ccg ctc att gcg aac gaa tca gat tgt ata gac atc gtt gat cca ttc    720
Pro Leu Ile Ala Asn Glu Ser Asp Cys Ile Asp Ile Val Asp Pro Phe
225             230             235             240

tta acc cta aaa ggg ctg gaa ttg att tat gaa aga aac cgc gta gga    768
Leu Thr Leu Lys Gly Leu Glu Leu Ile Tyr Glu Arg Asn Arg Val Gly
            245             250             255

agt gta tag                                                        777
Ser Val


<210> 20
<211> 960
<212> DNA
<213> Bacillus subtilis

<220>
<221> CDS
<222> (1)..(957)

<400> 20
gtg aaa aat aaa gaa ctt aac cta cat act tta tat aca cag cac aat    48
Met Lys Asn Lys Glu Leu Asn Leu His Thr Leu Tyr Thr Gln His Asn
 1               5              10              15

cgg gag tct tgg tct ggt ttt ggg ggg cat ttg tcg att gct gta tct    96
Arg Glu Ser Trp Ser Gly Phe Gly Gly His Leu Ser Ile Ala Val Ser
            20              25              30

gaa gaa gag gca aaa gct gtg gaa gga ttg aat gat tat cta tct gtt    144
Glu Glu Glu Ala Lys Ala Val Glu Gly Leu Asn Asp Tyr Leu Ser Val
            35              40              45

gaa gaa gtg gag acg atc tat att ccg ctt gtt cgc ttg ctt cat tta    192
Glu Glu Val Glu Thr Ile Tyr Ile Pro Leu Val Arg Leu Leu His Leu
        50              55              60

cat gtc aag tct gcg gct gaa cgc aat aag cat gtc aat gtt ttt ttg    240
His Val Lys Ser Ala Ala Glu Arg Asn Lys His Val Asn Val Phe Leu
65              70              75              80

aag cac cca cat tca gcc aaa att ccg ttt att atc ggc att gcc ggc    288
Lys His Pro His Ser Ala Lys Ile Pro Phe Ile Ile Gly Ile Ala Gly
                85              90              95
```

```
agt gtc gca gtc gga aaa agc acg acg gcg cgg atc ttg cag aag ctg    336
Ser Val Ala Val Gly Lys Ser Thr Thr Ala Arg Ile Leu Gln Lys Leu
            100             105             110

ctt tcg cgt ttg cct gac cgt cca aaa gtg agc ctt atc acg aca gat    384
Leu Ser Arg Leu Pro Asp Arg Pro Lys Val Ser Leu Ile Thr Thr Asp
            115             120             125

ggt ttt tta ttt cct act gcc gag ctg aaa aag aaa aat atg atg tca    432
Gly Phe Leu Phe Pro Thr Ala Glu Leu Lys Lys Lys Asn Met Met Ser
            130             135             140

aga aaa gga ttt cct gaa agc tat gat gta aag gcg ctg ctc gaa ttt    480
Arg Lys Gly Phe Pro Glu Ser Tyr Asp Val Lys Ala Leu Leu Glu Phe
145             150             155             160

ttg aat gac tta aaa tca gga aag gac agc gta aag gcc ccg gtg tat    528
Leu Asn Asp Leu Lys Ser Gly Lys Asp Ser Val Lys Ala Pro Val Tyr
            165             170             175

tcc cat cta acc tat gac cgc gag gaa ggt gtg ttc gag gtt gta gaa    576
Ser His Leu Thr Tyr Asp Arg Glu Glu Gly Val Phe Glu Val Val Glu
            180             185             190

cag gcg gat att gtg att att gaa ggc att aat gtt ctt cag tcg ccc    624
Gln Ala Asp Ile Val Ile Ile Glu Gly Ile Asn Val Leu Gln Ser Pro
            195             200             205

acc ttg gag gat gac cgg gaa aac ccg cgt att ttt gtt tcc gat ttc    672
Thr Leu Glu Asp Asp Arg Glu Asn Pro Arg Ile Phe Val Ser Asp Phe
            210             215             220

ttt gat ttt tcg att tat gtg gat gcg gag gaa agc cgg att ttc act    720
Phe Asp Phe Ser Ile Tyr Val Asp Ala Glu Glu Ser Arg Ile Phe Thr
225             230             235             240

tgg tat tta gag cgt ttt cgc ctg ctt cgg gaa aca gct ttt caa aat    768
Trp Tyr Leu Glu Arg Phe Arg Leu Leu Arg Glu Thr Ala Phe Gln Asn
            245             250             255

cct gat tca tat ttt cat aaa ttt aaa gac ttg tcc gat cag gag gct    816
Pro Asp Ser Tyr Phe His Lys Phe Lys Asp Leu Ser Asp Gln Glu Ala
            260             265             270

gac gag atg gca gcc tcg att tgg gag agt gtc aac cgg ccg aat tta    864
Asp Glu Met Ala Ala Ser Ile Trp Glu Ser Val Asn Arg Pro Asn Leu
            275             280             285

tat gaa aat att ttg cca act aaa ttc agg tca gat ctc att ttg cgt    912
Tyr Glu Asn Ile Leu Pro Thr Lys Phe Arg Ser Asp Leu Ile Leu Arg
            290             295             300

aag gga gac ggg cat aag gtc gag gaa gtg ttg gta agg agg gta tga    960
Lys Gly Asp Gly His Lys Val Glu Glu Val Leu Val Arg Arg Val
305             310             315
```

<210> 21
<211> 882
<212> DNA
<213> Bacillus subtilis

```
<220>
<221> CDS
<222> (1)..(879)

<400> 21
ttg tcg att gct gta tct gaa gaa gag gca aaa gct gtg gaa gga ttg      48
Met Ser Ile Ala Val Ser Glu Glu Glu Ala Lys Ala Val Glu Gly Leu
 1               5                  10                  15

aat gat tat cta tct gtt gaa gaa gtg gag acg atc tat att ccg ctt      96
Asn Asp Tyr Leu Ser Val Glu Glu Val Glu Thr Ile Tyr Ile Pro Leu
            20                  25                  30

gtt cgc ttg ctt cat tta cat gtc aag tct gcg gct gaa cgc aat aag     144
Val Arg Leu Leu His Leu His Val Lys Ser Ala Ala Glu Arg Asn Lys
        35                  40                  45

cat gtc aat gtt ttt ttg aag cac cca cat tca gcc aaa att ccg ttt     192
His Val Asn Val Phe Leu Lys His Pro His Ser Ala Lys Ile Pro Phe
    50                  55                  60

att atc ggc att gcc ggc agt gtc gca gtc gga aaa agc acg acg gcg     240
Ile Ile Gly Ile Ala Gly Ser Val Ala Val Gly Lys Ser Thr Thr Ala
65                  70                  75                  80

cgg atc ttg cag aag ctg ctt tcg cgt ttg cct gac cgt cca aaa gtg     288
Arg Ile Leu Gln Lys Leu Leu Ser Arg Leu Pro Asp Arg Pro Lys Val
                85                  90                  95

agc ctt atc acg aca gat ggt ttt tta ttt cct act gcc gag ctg aaa     336
Ser Leu Ile Thr Thr Asp Gly Phe Leu Phe Pro Thr Ala Glu Leu Lys
                100                 105                 110

aag aaa aat atg atg tca aga aaa gga ttt cct gaa agc tat gat gta     384
Lys Lys Asn Met Met Ser Arg Lys Gly Phe Pro Glu Ser Tyr Asp Val
            115                 120                 125

aag gcg ctg ctc gaa ttt ttg aat gac tta aaa tca gga aag gac agc     432
Lys Ala Leu Leu Glu Phe Leu Asn Asp Leu Lys Ser Gly Lys Asp Ser
        130                 135                 140

gta aag gcc ccg gtg tat tcc cat cta acc tat gac cgc gag gaa ggt     480
Val Lys Ala Pro Val Tyr Ser His Leu Thr Tyr Asp Arg Glu Glu Gly
145                 150                 155                 160

gtg ttc gag gtt gta gaa cag gcg gat att gtg att att gaa ggc att     528
Val Phe Glu Val Val Glu Gln Ala Asp Ile Val Ile Ile Glu Gly Ile
                165                 170                 175

aat gtt ctt cag tcg ccc acc ttg gag gat gac cgg gaa aac ccg cgt     576
Asn Val Leu Gln Ser Pro Thr Leu Glu Asp Asp Arg Glu Asn Pro Arg
                180                 185                 190

att ttt gtt tcc gat ttc ttt gat ttt tcg att tat gtg gat gcg gag     624
Ile Phe Val Ser Asp Phe Phe Asp Phe Ser Ile Tyr Val Asp Ala Glu
                195                 200                 205

gaa agc cgg att ttc act tgg tat tta gag cgt ttt cgc ctg ctt cgg     672
Glu Ser Arg Ile Phe Thr Trp Tyr Leu Glu Arg Phe Arg Leu Leu Arg
        210                 215                 220
```

```
gaa aca gct ttt caa aat cct gat tca tat ttt cat aaa ttt aaa gac   720
Glu Thr Ala Phe Gln Asn Pro Asp Ser Tyr Phe His Lys Phe Lys Asp
225                 230                 235                 240

ttg tcc gat cag gag gct gac gag atg gca gcc tcg att tgg gag agt   768
Leu Ser Asp Gln Glu Ala Asp Glu Met Ala Ala Ser Ile Trp Glu Ser
                245                 250                 255

gtc aac cgg ccg aat tta tat gaa aat att ttg cca act aaa ttc agg   816
Val Asn Arg Pro Asn Leu Tyr Glu Asn Ile Leu Pro Thr Lys Phe Arg
                260                 265                 270

tca gat ctc att ttg cgt aag gga gac ggg cat aag gtc gag gaa gtg   864
Ser Asp Leu Ile Leu Arg Lys Gly Asp Gly His Lys Val Glu Glu Val
                275                 280                 285

ttg gta agg agg gta tga                                           882
Leu Val Arg Arg Val
            290


<210> 22
<211> 846
<212> DNA
<213> Bacillus subtilis

<220>
<221> CDS
<222> (1)..(843)

<400> 22
gtg gaa gga ttg aat gat tat cta tct gtt gaa gaa gtg gag acg atc    48
Met Glu Gly Leu Asn Asp Tyr Leu Ser Val Glu Glu Val Glu Thr Ile
 1               5                  10                  15

tat att ccg ctt gtt cgc ttg ctt cat tta cat gtc aag tct gcg gct    96
Tyr Ile Pro Leu Val Arg Leu Leu His Leu His Val Lys Ser Ala Ala
                20                  25                  30

gaa cgc aat aag cat gtc aat gtt ttt ttg aag cac cca cat tca gcc   144
Glu Arg Asn Lys His Val Asn Val Phe Leu Lys His Pro His Ser Ala
            35                  40                  45

aaa att ccg ttt att atc ggc att gcc ggc agt gtc gca gtc gga aaa   192
Lys Ile Pro Phe Ile Ile Gly Ile Ala Gly Ser Val Ala Val Gly Lys
        50                  55                  60

agc acg acg gcg cgg atc ttg cag aag ctg ctt tcg cgt ttg cct gac   240
Ser Thr Thr Ala Arg Ile Leu Gln Lys Leu Leu Ser Arg Leu Pro Asp
65                  70                  75                  80

cgt cca aaa gtg agc ctt atc acg aca gat ggt ttt tta ttt cct act   288
Arg Pro Lys Val Ser Leu Ile Thr Thr Asp Gly Phe Leu Phe Pro Thr
                85                  90                  95

gcc gag ctg aaa aag aaa aat atg atg tca aga aaa gga ttt cct gaa   336
Ala Glu Leu Lys Lys Lys Asn Met Met Ser Arg Lys Gly Phe Pro Glu
                100                 105                 110

agc tat gat gta aag gcg ctg ctc gaa ttt ttg aat gac tta aaa tca   384
```

```
Ser Tyr Asp Val Lys Ala Leu Leu Glu Phe Leu Asn Asp Leu Lys Ser
        115             120                 125

gga aag gac agc gta aag gcc ccg gtg tat tcc cat cta acc tat gac    432
Gly Lys Asp Ser Val Lys Ala Pro Val Tyr Ser His Leu Thr Tyr Asp
        130             135                 140

cgc gag gaa ggt gtg ttc gag gtt gta gaa cag gcg gat att gtg att    480
Arg Glu Glu Gly Val Phe Glu Val Val Glu Gln Ala Asp Ile Val Ile
145             150                 155                 160

att gaa ggc att aat gtt ctt cag tcg ccc acc ttg gag gat gac cgg    528
Ile Glu Gly Ile Asn Val Leu Gln Ser Pro Thr Leu Glu Asp Asp Arg
                165                 170                 175

gaa aac ccg cgt att ttt gtt tcc gat ttc ttt gat ttt tcg att tat    576
Glu Asn Pro Arg Ile Phe Val Ser Asp Phe Phe Asp Phe Ser Ile Tyr
                180                 185                 190

gtg gat gcg gag gaa agc cgg att ttc act tgg tat tta gag cgt ttt    624
Val Asp Ala Glu Glu Ser Arg Ile Phe Thr Trp Tyr Leu Glu Arg Phe
            195                 200                 205

cgc ctg ctt cgg gaa aca gct ttt caa aat cct gat tca tat ttt cat    672
Arg Leu Leu Arg Glu Thr Ala Phe Gln Asn Pro Asp Ser Tyr Phe His
    210                 215                 220

aaa ttt aaa gac ttg tcc gat cag gag gct gac gag atg gca gcc tcg    720
Lys Phe Lys Asp Leu Ser Asp Gln Glu Ala Asp Glu Met Ala Ala Ser
225             230                 235                 240

att tgg gag agt gtc aac cgg ccg aat tta tat gaa aat att ttg cca    768
Ile Trp Glu Ser Val Asn Arg Pro Asn Leu Tyr Glu Asn Ile Leu Pro
                245                 250                 255

act aaa ttc agg tca gat ctc att ttg cgt aag gga gac ggg cat aag    816
Thr Lys Phe Arg Ser Asp Leu Ile Leu Arg Lys Gly Asp Gly His Lys
                260                 265                 270

gtc gag gaa gtg ttg gta agg agg gta tga                            846
Val Glu Glu Val Leu Val Arg Arg Val
            275                 280
```

```
<210> 23
<211> 831
<212> DNA
<213> Bacillus subtilis

<220>
<221> CDS
<222> (1)..(831)

<400> 23
atg aaa aca aaa ctg gat ttt cta aaa atg aag gag tct gaa gaa ccg    48
Met Lys Thr Lys Leu Asp Phe Leu Lys Met Lys Glu Ser Glu Glu Pro
  1             5                  10                  15

att gtc atg ctg acc gct tat gat tat ccg gca gct aaa ctt gct gaa    96
Ile Val Met Leu Thr Ala Tyr Asp Tyr Pro Ala Ala Lys Leu Ala Glu
                20                  25                  30
```

```
caa gcg gga gtt gac atg att tta gtc ggt gat tca ctt gga atg gtc    144
Gln Ala Gly Val Asp Met Ile Leu Val Gly Asp Ser Leu Gly Met Val
         35                  40                  45

gtc ctc ggc ctt gat tca act gtc ggt gtg aca gtt gcg gac atg atc    192
Val Leu Gly Leu Asp Ser Thr Val Gly Val Thr Val Ala Asp Met Ile
     50                  55                  60

cat cat aca aaa gcc gtt aaa agg ggt gcg ccg aat acc ttt att gtg    240
His His Thr Lys Ala Val Lys Arg Gly Ala Pro Asn Thr Phe Ile Val
65                  70                  75                  80

aca gat atg ccg ttt atg tct tat cac ctg tct aag gaa gat acg ctg    288
Thr Asp Met Pro Phe Met Ser Tyr His Leu Ser Lys Glu Asp Thr Leu
                 85                  90                  95

aaa aat gca gcg gct atc gtt cag gaa agc gga gct gac gca ctg aag    336
Lys Asn Ala Ala Ala Ile Val Gln Glu Ser Gly Ala Asp Ala Leu Lys
            100                 105                 110

ctt gag ggc gga gaa ggc gtg ttt gaa tcc att cgc gca ttg acg ctt    384
Leu Glu Gly Gly Glu Gly Val Phe Glu Ser Ile Arg Ala Leu Thr Leu
         115                 120                 125

gga ggc att cca gta gtc agt cac tta ggt ttg aca ccg cag tca gtc    432
Gly Gly Ile Pro Val Val Ser His Leu Gly Leu Thr Pro Gln Ser Val
         130                 135                 140

ggc gta ctg ggc ggc tat aaa gta cag ggc aaa gac gaa caa agc gcc    480
Gly Val Leu Gly Gly Tyr Lys Val Gln Gly Lys Asp Glu Gln Ser Ala
145                 150                 155                 160

aaa aaa tta ata gaa gac agt ata aaa tgc gaa gaa gca gga gct atg    528
Lys Lys Leu Ile Glu Asp Ser Ile Lys Cys Glu Glu Ala Gly Ala Met
                 165                 170                 175

atg ctt gtg ctg gaa tgt gtg ccg gca gaa ctc aca gcc aaa att gcc    576
Met Leu Val Leu Glu Cys Val Pro Ala Glu Leu Thr Ala Lys Ile Ala
         180                 185                 190

gag acg cta agc ata ccg gtc att gga atc ggg gct ggt gtg aaa gcg    624
Glu Thr Leu Ser Ile Pro Val Ile Gly Ile Gly Ala Gly Val Lys Ala
         195                 200                 205

gac gga caa gtt ctc gtt tat cat gat att atc ggc cac ggt gtt gag    672
Asp Gly Gln Val Leu Val Tyr His Asp Ile Ile Gly His Gly Val Glu
210                 215                 220

aga aca cct aaa ttt gta aag caa tat acg cgc att gat gaa acc atc    720
Arg Thr Pro Lys Phe Val Lys Gln Tyr Thr Arg Ile Asp Glu Thr Ile
225                 230                 235                 240

gaa aca gca atc agc gga tat gtt cag gat gta aga cat cgt gct ttc    768
Glu Thr Ala Ile Ser Gly Tyr Val Gln Asp Val Arg His Arg Ala Phe
                 245                 250                 255

cct gaa caa aag cat tcc ttt caa atg aac cag aca gtg ctt gac ggc    816
Pro Glu Gln Lys His Ser Phe Gln Met Asn Gln Thr Val Leu Asp Gly
                 260                 265                 270
```

```
ttg tac ggg gga aaa                                              831
Leu Tyr Gly Gly Lys
        275


<210> 24
<211> 277
<212> PRT
<213> Bacillus subtilis

<400> 24
Met Lys Thr Lys Leu Asp Phe Leu Lys Met Lys Glu Ser Glu Glu Pro
 1               5               10                  15

Ile Val Met Leu Thr Ala Tyr Asp Tyr Pro Ala Ala Lys Leu Ala Glu
            20              25                  30

Gln Ala Gly Val Asp Met Ile Leu Val Gly Asp Ser Leu Gly Met Val
        35              40              45

Val Leu Gly Leu Asp Ser Thr Val Gly Val Thr Val Ala Asp Met Ile
    50              55              60

His His Thr Lys Ala Val Lys Arg Gly Ala Pro Asn Thr Phe Ile Val
65              70                  75                  80

Thr Asp Met Pro Phe Met Ser Tyr His Leu Ser Lys Glu Asp Thr Leu
            85              90                  95

Lys Asn Ala Ala Ala Ile Val Gln Glu Ser Gly Ala Asp Ala Leu Lys
            100             105             110

Leu Glu Gly Gly Glu Gly Val Phe Glu Ser Ile Arg Ala Leu Thr Leu
        115             120             125

Gly Gly Ile Pro Val Val Ser His Leu Gly Leu Thr Pro Gln Ser Val
    130             135             140

Gly Val Leu Gly Gly Tyr Lys Val Gln Gly Lys Asp Glu Gln Ser Ala
145             150             155             160

Lys Lys Leu Ile Glu Asp Ser Ile Lys Cys Glu Glu Ala Gly Ala Met
            165             170             175

Met Leu Val Leu Glu Cys Val Pro Ala Glu Leu Thr Ala Lys Ile Ala
        180             185             190

Glu Thr Leu Ser Ile Pro Val Ile Gly Ile Gly Ala Gly Val Lys Ala
        195             200             205

Asp Gly Gln Val Leu Val Tyr His Asp Ile Ile Gly His Gly Val Glu
    210             215             220

Arg Thr Pro Lys Phe Val Lys Gln Tyr Thr Arg Ile Asp Glu Thr Ile
225             230             235             240

Glu Thr Ala Ile Ser Gly Tyr Val Gln Asp Val Arg His Arg Ala Phe
            245             250             255

Pro Glu Gln Lys His Ser Phe Gln Met Asn Gln Thr Val Leu Asp Gly
        260             265             270
```

Leu Tyr Gly Gly Lys
         275

<210> 25
<211> 858
<212> DNA
<213> Bacillus subtilis

<220>
<221> CDS
<222> (1)..(858)

<400> 25
atg aga cag att act gat att tca cag ctg aaa gaa gcc ata aaa caa   48
Met Arg Gln Ile Thr Asp Ile Ser Gln Leu Lys Glu Ala Ile Lys Gln
  1   5   10   15

tac cat tca gag ggc aag tca atc gga ttt gtt ccg acg atg ggg ttt   96
Tyr His Ser Glu Gly Lys Ser Ile Gly Phe Val Pro Thr Met Gly Phe
                 20   25   30

ctg cat gag ggg cat tta acc tta gca gac aaa gca aga caa gaa aac   144
Leu His Glu Gly His Leu Thr Leu Ala Asp Lys Ala Arg Gln Glu Asn
             35   40   45

gac gcc gtt att atg agt att ttt gtg aat cct gca caa ttc ggc cct   192
Asp Ala Val Ile Met Ser Ile Phe Val Asn Pro Ala Gln Phe Gly Pro
         50   55   60

aat gaa gat ttt gaa gca tat ccg cgc gat att gag cgg gat gca gct   240
Asn Glu Asp Phe Glu Ala Tyr Pro Arg Asp Ile Glu Arg Asp Ala Ala
 65   70   75   80

ctt gca gaa aac gcc gga gtc gat att ctt ttt acg cca gat gct cat   288
Leu Ala Glu Asn Ala Gly Val Asp Ile Leu Phe Thr Pro Asp Ala His
                 85   90   95

gat atg tat ccc ggt gaa aag aat gtc acg att cat gta gaa aga cgc   336
Asp Met Tyr Pro Gly Glu Lys Asn Val Thr Ile His Val Glu Arg Arg
                100   105   110

aca gac gtg tta tgc ggg cgc tca aga gaa gga cat ttt gac ggg gtc   384
Thr Asp Val Leu Cys Gly Arg Ser Arg Glu Gly His Phe Asp Gly Val
             115   120   125

gcg atc gta ctg acg aag ctt ttc aat cta gtc aag ccg act cgt gcc   432
Ala Ile Val Leu Thr Lys Leu Phe Asn Leu Val Lys Pro Thr Arg Ala
         130   135   140

tat ttc ggt tta aaa gat gcg cag cag gta gct gtt gtt gat ggg tta   480
Tyr Phe Gly Leu Lys Asp Ala Gln Gln Val Ala Val Val Asp Gly Leu
145   150   155   160

atc agc gac ttc ttc atg gat att gaa ttg gtt cct gtc gat acg gtc   528
Ile Ser Asp Phe Phe Met Asp Ile Glu Leu Val Pro Val Asp Thr Val
                165   170   175

aga gag gaa gac ggc tta gcc aaa agc tct cgc aat gta tac tta aca   576
Arg Glu Glu Asp Gly Leu Ala Lys Ser Ser Arg Asn Val Tyr Leu Thr

```
                 180                    185                    190

gct gag gaa aga aaa gaa gcg cct aag ctg tat cgg gcc ctt caa aca    624
Ala Glu Glu Arg Lys Glu Ala Pro Lys Leu Tyr Arg Ala Leu Gln Thr
            195                    200                    205

agt gcg gaa ctt gtc caa gcc ggt gaa aga gat cct gaa gcg gtg ata    672
Ser Ala Glu Leu Val Gln Ala Gly Glu Arg Asp Pro Glu Ala Val Ile
    210                    215                    220

aaa gct gca aaa gat atc att gaa acg act agc gga acc ata gac tat    720
Lys Ala Ala Lys Asp Ile Ile Glu Thr Thr Ser Gly Thr Ile Asp Tyr
225                    230                    235                    240

gta gag ctt tat tcc tat ccg gaa ctc gag cct gtg aat gaa att gct    768
Val Glu Leu Tyr Ser Tyr Pro Glu Leu Glu Pro Val Asn Glu Ile Ala
                    245                    250                    255

gga aag atg att ctc gct gtt gca gtt gct ttt tca aaa gcg cgt tta    816
Gly Lys Met Ile Leu Ala Val Ala Val Ala Phe Ser Lys Ala Arg Leu
                260                    265                    270

ata gat aat atc att att gat att cga gaa atg gag aga ata            858
Ile Asp Asn Ile Ile Ile Asp Ile Arg Glu Met Glu Arg Ile
                275                    280                    285
```

<210> 26
<211> 286
<212> PRT
<213> Bacillus subtilis

<400> 26

```
Met Arg Gln Ile Thr Asp Ile Ser Gln Leu Lys Glu Ala Ile Lys Gln
 1               5                  10                  15

Tyr His Ser Glu Gly Lys Ser Ile Gly Phe Val Pro Thr Met Gly Phe
            20                  25                  30

Leu His Glu Gly His Leu Thr Leu Ala Asp Lys Ala Arg Gln Glu Asn
        35                  40                  45

Asp Ala Val Ile Met Ser Ile Phe Val Asn Pro Ala Gln Phe Gly Pro
    50                  55                  60

Asn Glu Asp Phe Glu Ala Tyr Pro Arg Asp Ile Glu Arg Asp Ala Ala
65                  70                  75                  80

Leu Ala Glu Asn Ala Gly Val Asp Ile Leu Phe Thr Pro Asp Ala His
                85                  90                  95

Asp Met Tyr Pro Gly Glu Lys Asn Val Thr Ile His Val Glu Arg Arg
                100                 105                 110

Thr Asp Val Leu Cys Gly Arg Ser Arg Glu Gly His Phe Asp Gly Val
            115                 120                 125

Ala Ile Val Leu Thr Lys Leu Phe Asn Leu Val Lys Pro Thr Arg Ala
    130                 135                 140

Tyr Phe Gly Leu Lys Asp Ala Gln Gln Val Ala Val Val Asp Gly Leu
```

```
145                      150                    155                      160

Ile Ser Asp Phe Phe Met Asp Ile Glu Leu Val Pro Val Asp Thr Val
              165               170               175

Arg Glu Glu Asp Gly Leu Ala Lys Ser Ser Arg Asn Val Tyr Leu Thr
          180               185               190

Ala Glu Glu Arg Lys Glu Ala Pro Lys Leu Tyr Arg Ala Leu Gln Thr
          195               200               205

Ser Ala Glu Leu Val Gln Ala Gly Glu Arg Asp Pro Glu Ala Val Ile
    210               215               220

Lys Ala Ala Lys Asp Ile Ile Glu Thr Thr Ser Gly Thr Ile Asp Tyr
225               230               235               240

Val Glu Leu Tyr Ser Tyr Pro Glu Leu Glu Pro Val Asn Glu Ile Ala
              245               250               255

Gly Lys Met Ile Leu Ala Val Ala Val Ala Phe Ser Lys Ala Arg Leu
              260               265               270

Ile Asp Asn Ile Ile Ile Asp Ile Arg Glu Met Glu Arg Ile
          275               280               285
```

<210> 27
<211> 381
<212> DNA
<213> Bacillus subtilis

<220>
<221> CDS
<222> (1)..(381)

<400> 27

```
atg tat cga aca atg atg agc ggc aaa ctt cac agg gca act gtt acg   48
Met Tyr Arg Thr Met Met Ser Gly Lys Leu His Arg Ala Thr Val Thr
 1               5               10                    15

gaa gca aac ctg aac tat gtg gga agc att aca att gat gaa gat ctc   96
Glu Ala Asn Leu Asn Tyr Val Gly Ser Ile Thr Ile Asp Glu Asp Leu
              20                  25                  30

att gat gct gtg gga atg ctt cct aat gaa aaa gta caa att gtg aat  144
Ile Asp Ala Val Gly Met Leu Pro Asn Glu Lys Val Gln Ile Val Asn
          35                  40                  45

aat aat aat gga gca cgt ctt gaa acg tat att att cct ggt aaa cgg  192
Asn Asn Asn Gly Ala Arg Leu Glu Thr Tyr Ile Ile Pro Gly Lys Arg
      50                  55                  60

gga agc ggc gtc ata tgc tta aac ggt gca gcc gca cgc ctt gtg cag  240
Gly Ser Gly Val Ile Cys Leu Asn Gly Ala Ala Ala Arg Leu Val Gln
 65                  70                  75                  80

gaa gga gat aag gtc att att att tcc tac aaa atg atg tct gat caa  288
Glu Gly Asp Lys Val Ile Ile Ile Ser Tyr Lys Met Met Ser Asp Gln
              85                  90                  95
```

```
gaa gcg gca agc cat gag ccg aaa gtg gct gtt ctg aat gat caa aac    336
Glu Ala Ala Ser His Glu Pro Lys Val Ala Val Leu Asn Asp Gln Asn
            100                 105                 110

aaa att gaa caa atg ctg ggg aac gaa cca gcc cgt aca att ttg        381
Lys Ile Glu Gln Met Leu Gly Asn Glu Pro Ala Arg Thr Ile Leu
            115                 120                 125
```

<210> 28
<211> 127
<212> PRT
<213> Bacillus subtilis

<400> 28
```
Met Tyr Arg Thr Met Met Ser Gly Lys Leu His Arg Ala Thr Val Thr
 1               5                  10                  15

Glu Ala Asn Leu Asn Tyr Val Gly Ser Ile Thr Ile Asp Glu Asp Leu
            20                  25                  30

Ile Asp Ala Val Gly Met Leu Pro Asn Glu Lys Val Gln Ile Val Asn
            35                  40                  45

Asn Asn Asn Gly Ala Arg Leu Glu Thr Tyr Ile Ile Pro Gly Lys Arg
     50                  55                  60

Gly Ser Gly Val Ile Cys Leu Asn Gly Ala Ala Ala Arg Leu Val Gln
 65                  70                  75                  80

Glu Gly Asp Lys Val Ile Ile Ile Ser Tyr Lys Met Met Ser Asp Gln
                85                  90                  95

Glu Ala Ala Ser His Glu Pro Lys Val Ala Val Leu Asn Asp Gln Asn
            100                 105                 110

Lys Ile Glu Gln Met Leu Gly Asn Glu Pro Ala Arg Thr Ile Leu
            115                 120                 125
```

<210> 29
<211> 894
<212> DNA
<213> Bacillus subtilis

<220>
<221> CDS
<222> (1)..(894)

<400> 29
```
atg aaa att gga att atc ggc gga ggc tcc gtt ggt ctt tta tgc gcc     48
Met Lys Ile Gly Ile Ile Gly Gly Gly Ser Val Gly Leu Leu Cys Ala
 1               5                  10                  15

tat tat ttg tca ctt tat cac gac gtg act gtt gtg acg agg cgg caa     96
Tyr Tyr Leu Ser Leu Tyr His Asp Val Thr Val Val Thr Arg Arg Gln
            20                  25                  30

gaa cag gct gcg gcc att cag tct gaa gga atc cgg ctt tat aaa ggc    144
Glu Gln Ala Ala Ala Ile Gln Ser Glu Gly Ile Arg Leu Tyr Lys Gly
            35                  40                  45
```

```
ggg gag gaa ttc agg gct gat tgc agt gcg gac acg agt atc aat tcg    192
Gly Glu Glu Phe Arg Ala Asp Cys Ser Ala Asp Thr Ser Ile Asn Ser
    50              55                  60

gac ttt gac ctg ctt gtc gtg aca gtg aag cag cat cag ctt caa tct    240
Asp Phe Asp Leu Leu Val Val Thr Val Lys Gln His Gln Leu Gln Ser
65              70                  75                  80

gtt ttt tcg tcg ctt gaa cga atc ggg aag acg aat ata tta ttt ttg    288
Val Phe Ser Ser Leu Glu Arg Ile Gly Lys Thr Asn Ile Leu Phe Leu
                85                  90                  95

caa aac ggc atg ggg cat atc cac gac cta aaa gac tgg cac gtt ggc    336
Gln Asn Gly Met Gly His Ile His Asp Leu Lys Asp Trp His Val Gly
            100                 105                 110

cat tcc att tat gtt gga atc gtt gag cac gga gct gta aga aaa tcg    384
His Ser Ile Tyr Val Gly Ile Val Glu His Gly Ala Val Arg Lys Ser
            115                 120                 125

gat aca gct gtt gat cat aca ggc cta ggt gcg ata aaa tgg agc gcg    432
Asp Thr Ala Val Asp His Thr Gly Leu Gly Ala Ile Lys Trp Ser Ala
    130                 135                 140

ttc gac gat gct gaa cca gac cgg ctg aac atc ttg ttt cag cat aac    480
Phe Asp Asp Ala Glu Pro Asp Arg Leu Asn Ile Leu Phe Gln His Asn
145                 150                 155                 160

cat tcg gat ttt ccg att tat tat gag acg gat tgg tac cgt ctg ctg    528
His Ser Asp Phe Pro Ile Tyr Tyr Glu Thr Asp Trp Tyr Arg Leu Leu
                165                 170                 175

acg ggc aag ctg att gta aat gcg tgt att aat cct tta act gcg tta    576
Thr Gly Lys Leu Ile Val Asn Ala Cys Ile Asn Pro Leu Thr Ala Leu
            180                 185                 190

ttg caa gtg aaa aat gga gaa ctg ctg aca acg cca gct tat ctg gct    624
Leu Gln Val Lys Asn Gly Glu Leu Leu Thr Thr Pro Ala Tyr Leu Ala
            195                 200                 205

ttt atg aag ctg gta ttt cag gag gca tgc cgc att tta aaa ctt gaa    672
Phe Met Lys Leu Val Phe Gln Glu Ala Cys Arg Ile Leu Lys Leu Glu
    210                 215                 220

aat gaa gaa aag gct tgg gag cgg gtt cag gcc gtt tgt ggg caa acg    720
Asn Glu Glu Lys Ala Trp Glu Arg Val Gln Ala Val Cys Gly Gln Thr
225                 230                 235                 240

aaa gag aat cgt tca tca atg ctg gtt gac gtc att gga ggc cgg cag    768
Lys Glu Asn Arg Ser Ser Met Leu Val Asp Val Ile Gly Gly Arg Gln
                245                 250                 255

acg gaa gct gac gcc att atc gga tac tta ttg aag gaa gca agt ctt    816
Thr Glu Ala Asp Ala Ile Ile Gly Tyr Leu Leu Lys Glu Ala Ser Leu
            260                 265                 270

caa ggt ctt gat gcc gtc cac cta gag ttt tta tat ggc agc atc aaa    864
Gln Gly Leu Asp Ala Val His Leu Glu Phe Leu Tyr Gly Ser Ile Lys
            275                 280                 285
```

```
gca ttg gag cga aat aca aac aaa gtc ttt                                      894
Ala Leu Glu Arg Asn Thr Asn Lys Val Phe
    290                 295
```

<210> 30
<211> 298
<212> PRT
<213> Bacillus subtilis

<400> 30

```
Met Lys Ile Gly Ile Ile Gly Gly Gly Ser Val Gly Leu Leu Cys Ala
 1               5                  10                  15

Tyr Tyr Leu Ser Leu Tyr His Asp Val Thr Val Val Thr Arg Arg Gln
            20              25                  30

Glu Gln Ala Ala Ala Ile Gln Ser Glu Gly Ile Arg Leu Tyr Lys Gly
        35                  40                  45

Gly Glu Glu Phe Arg Ala Asp Cys Ser Ala Asp Thr Ser Ile Asn Ser
    50                  55                  60

Asp Phe Asp Leu Leu Val Val Thr Val Lys Gln His Gln Leu Gln Ser
65                  70                  75                  80

Val Phe Ser Ser Leu Glu Arg Ile Gly Lys Thr Asn Ile Leu Phe Leu
                85                  90                  95

Gln Asn Gly Met Gly His Ile His Asp Leu Lys Asp Trp His Val Gly
               100                 105                 110

His Ser Ile Tyr Val Gly Ile Val Glu His Gly Ala Val Arg Lys Ser
       115                 120                 125

Asp Thr Ala Val Asp His Thr Gly Leu Gly Ala Ile Lys Trp Ser Ala
   130                 135                 140

Phe Asp Asp Ala Glu Pro Asp Arg Leu Asn Ile Leu Phe Gln His Asn
145                 150                 155                 160

His Ser Asp Phe Pro Ile Tyr Tyr Glu Thr Asp Trp Tyr Arg Leu Leu
               165                 170                 175

Thr Gly Lys Leu Ile Val Asn Ala Cys Ile Asn Pro Leu Thr Ala Leu
           180                 185                 190

Leu Gln Val Lys Asn Gly Glu Leu Leu Thr Thr Pro Ala Tyr Leu Ala
       195                 200                 205

Phe Met Lys Leu Val Phe Gln Glu Ala Cys Arg Ile Leu Lys Leu Glu
   210                 215                 220

Asn Glu Glu Lys Ala Trp Glu Arg Val Gln Ala Val Cys Gly Gln Thr
225                 230                 235                 240

Lys Glu Asn Arg Ser Ser Met Leu Val Asp Val Ile Gly Gly Arg Gln
               245                 250                 255

Thr Glu Ala Asp Ala Ile Ile Gly Tyr Leu Leu Lys Glu Ala Ser Leu
           260                 265                 270
```

Gln Gly Leu Asp Ala Val His Leu Glu Phe Leu Tyr Gly Ser Ile Lys
    275   .   280   285

Ala Leu Glu Arg Asn Thr Asn Lys Val Phe
    290   295

<210> 31
<211> 1725
<212> DNA
<213> Bacillus subtilis

<220>
<221> CDS
<222> (1)..(1722)

<400> 31
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atg ggg act aat gta cag gtg gat tca gca tct gcc gaa tgt aca cag    48
Met Gly Thr Asn Val Gln Val Asp Ser Ala Ser Ala Glu Cys Thr Gln
 1               5                  10                  15

acg atg agc gga gca tta atg ctg att gaa tca tta aaa aaa gag aaa    96
Thr Met Ser Gly Ala Leu Met Leu Ile Glu Ser Leu Lys Lys Glu Lys
                20                  25                  30

gta gaa atg atc ttc ggt tat ccg ggc ggg gct gtg ctt ccg att tac   144
Val Glu Met Ile Phe Gly Tyr Pro Gly Gly Ala Val Leu Pro Ile Tyr
            35                  40                  45

gat aag cta tac aat tca ggg ttg gta cat atc ctt ccc cgt cac gaa   192
Asp Lys Leu Tyr Asn Ser Gly Leu Val His Ile Leu Pro Arg His Glu
        50                  55                  60

caa gga gca att cat gca gcg gag gga tac gca agg gtc tcc gga aaa   240
Gln Gly Ala Ile His Ala Ala Glu Gly Tyr Ala Arg Val Ser Gly Lys
65                  70                  75                  80

ccg ggt gtc gtc att gcc acg tca ggg ccg gga gcg aca aac ctt gtt   288
Pro Gly Val Val Ile Ala Thr Ser Gly Pro Gly Ala Thr Asn Leu Val
                    85                  90                  95

aca ggc ctt gct gat gcc atg att gat tca ttg ccg tta gtc gtc ttt   336
Thr Gly Leu Ala Asp Ala Met Ile Asp Ser Leu Pro Leu Val Val Phe
                100                 105                 110

aca ggg cag gta gca acc tct gta atc ggg agc gat gca ttt cag gaa   384
Thr Gly Gln Val Ala Thr Ser Val Ile Gly Ser Asp Ala Phe Gln Glu
            115                 120                 125

gca gac att tta ggg att acg atg cca gta aca aaa cac agc tac cag   432
Ala Asp Ile Leu Gly Ile Thr Met Pro Val Thr Lys His Ser Tyr Gln
        130                 135                 140

gtt cgc cag ccg gaa gat ctg ccg cgc atc att aaa gaa gcg ttc cat   480
Val Arg Gln Pro Glu Asp Leu Pro Arg Ile Ile Lys Glu Ala Phe His
145                 150                 155                 160

att gca aca act gga aga ccc gga cct gta ttg att gat att ccg aaa   528
Ile Ala Thr Thr Gly Arg Pro Gly Pro Val Leu Ile Asp Ile Pro Lys
                165                 170                 175
```

```
gat gta gca aca att gaa gga gaa ttc agc tac gat cat gag atg aat    576
Asp Val Ala Thr Ile Glu Gly Glu Phe Ser Tyr Asp His Glu Met Asn
            180             185             190

ctc ccg gga tac cag ccg aca aca gag ccg aat tat ttg cag atc cgc    624
Leu Pro Gly Tyr Gln Pro Thr Thr Glu Pro Asn Tyr Leu Gln Ile Arg
            195             200             205

aag ctt gtg gaa gcc gtg agc agt gcg aaa aaa ccg gtg atc ctg gcg    672
Lys Leu Val Glu Ala Val Ser Ser Ala Lys Lys Pro Val Ile Leu Ala
            210             215             220

ggt gcg ggc gta ctg cac gga aaa gcg tca gaa gaa tta aaa aat tat    720
Gly Ala Gly Val Leu His Gly Lys Ala Ser Glu Glu Leu Lys Asn Tyr
225             230             235             240

gct gaa cag cag caa atc cct gtg gca cac acc ctt ttg ggg ctc gga    768
Ala Glu Gln Gln Gln Ile Pro Val Ala His Thr Leu Leu Gly Leu Gly
            245             250             255

ggc ttc ccg gct gac cat ccg ctt ttc cta ggg atg gcg gga atg cac    816
Gly Phe Pro Ala Asp His Pro Leu Phe Leu Gly Met Ala Gly Met His
            260             265             270

ggt act tat aca gcc aat atg gcc ctt cat gaa tgt gat cta tta atc    864
Gly Thr Tyr Thr Ala Asn Met Ala Leu His Glu Cys Asp Leu Leu Ile
            275             280             285

agt atc ggc gcc cgt ttt gat gac cgt gtc aca gga aac ctg aaa cac    912
Ser Ile Gly Ala Arg Phe Asp Asp Arg Val Thr Gly Asn Leu Lys His
            290             295             300

ttt gcc aga aac gca aag ata gcc cac atc gat att gat cca gct gaa    960
Phe Ala Arg Asn Ala Lys Ile Ala His Ile Asp Ile Asp Pro Ala Glu
305             310             315             320

atc gga aaa atc atg aaa aca cag att cct gta gtc gga gac agc aaa   1008
Ile Gly Lys Ile Met Lys Thr Gln Ile Pro Val Val Gly Asp Ser Lys
                325             330             335

att gtc ctg cag gag ctg atc aaa caa gac ggc aaa caa agc gat tca   1056
Ile Val Leu Gln Glu Leu Ile Lys Gln Asp Gly Lys Gln Ser Asp Ser
            340             345             350

agc gaa tgg aaa aaa cag ctc gca gaa tgg aaa gaa gag tat ccg ctc   1104
Ser Glu Trp Lys Lys Gln Leu Ala Glu Trp Lys Glu Glu Tyr Pro Leu
            355             360             365

tgg tat gta gat aat gaa gaa gaa ggt ttt aaa cct cag aaa ttg att   1152
Trp Tyr Val Asp Asn Glu Glu Glu Gly Phe Lys Pro Gln Lys Leu Ile
            370             375             380

gaa tat att cat caa ttt aca aaa gga gag gcc att gtc gca acg gat   1200
Glu Tyr Ile His Gln Phe Thr Lys Gly Glu Ala Ile Val Ala Thr Asp
385             390             395             400

gta ggc cag cat caa atg tgg tca gcg caa ttt tat ccg ttc caa aaa   1248
Val Gly Gln His Gln Met Trp Ser Ala Gln Phe Tyr Pro Phe Gln Lys
            405             410             415
```

```
gca gat aaa tgg gtc acg tca ggc gga ctt gga acg atg gga ttc ggt    1296
Ala Asp Lys Trp Val Thr Ser Gly Gly Leu Gly Thr Met Gly Phe Gly
            420                     425                 430

ctt ccg gcg gcg atc ggc gca cag ctg gcc gaa aaa gat gct act gtt    1344
Leu Pro Ala Ala Ile Gly Ala Gln Leu Ala Glu Lys Asp Ala Thr Val
            435                     440                 445

gtc gcg gtt gtc gga gac ggc gga ttc caa atg acg ctt caa gaa ctc    1392
Val Ala Val Val Gly Asp Gly Gly Phe Gln Met Thr Leu Gln Glu Leu
        450                     455                 460

gat gtt att cgc gaa tta aat ctt ccg gtc aag gta gtg att tta aat    1440
Asp Val Ile Arg Glu Leu Asn Leu Pro Val Lys Val Val Ile Leu Asn
465                     470                 475                 480

aac gct tgt ctc gga atg gtc aga cag tgg cag gaa att ttc tat gaa    1488
Asn Ala Cys Leu Gly Met Val Arg Gln Trp Gln Glu Ile Phe Tyr Glu
                485                     490                 495

gaa cgt tat tca gaa tct aaa ttc gct tct cag cct gac ttc gtc aaa    1536
Glu Arg Tyr Ser Glu Ser Lys Phe Ala Ser Gln Pro Asp Phe Val Lys
            500                     505                 510

ttg tcc gaa gca tac ggc att aaa ggc atc aga att tca tca gaa gcg    1584
Leu Ser Glu Ala Tyr Gly Ile Lys Gly Ile Arg Ile Ser Ser Glu Ala
            515                     520                 525

gaa gca aag gaa aag ctg gaa gag gca tta aca tca aga gaa cct gtt    1632
Glu Ala Lys Glu Lys Leu Glu Glu Ala Leu Thr Ser Arg Glu Pro Val
        530                     535                 540

gtc att gac gtg cgg gtt gcc agc gaa gaa aaa gta ttc ccg atg gtg    1680
Val Ile Asp Val Arg Val Ala Ser Glu Glu Lys Val Phe Pro Met Val
545                     550                     555                 560

gct ccg ggg aaa ggg ctg cat gaa atg gtg ggg gtg aaa cct tga       1725
Ala Pro Gly Lys Gly Leu His Glu Met Val Gly Val Lys Pro
            565                     570
```

```
<210> 32
<211> 574
<212> PRT
<213> Bacillus subtilis

<400> 32
Met Gly Thr Asn Val Gln Val Asp Ser Ala Ser Ala Glu Cys Thr Gln
 1               5                  10                  15

Thr Met Ser Gly Ala Leu Met Leu Ile Glu Ser Leu Lys Lys Glu Lys
            20                  25                  30

Val Glu Met Ile Phe Gly Tyr Pro Gly Gly Ala Val Leu Pro Ile Tyr
            35                  40                  45

Asp Lys Leu Tyr Asn Ser Gly Leu Val His Ile Leu Pro Arg His Glu
        50                  55                  60

Gln Gly Ala Ile His Ala Ala Glu Gly Tyr Ala Arg Val Ser Gly Lys
65                  70                  75                  80
```

Pro Gly Val Val Ile Ala Thr Ser Gly Pro Gly Ala Thr Asn Leu Val
                85   90   95

Thr Gly Leu Ala Asp Ala Met Ile Asp Ser Leu Pro Leu Val Val Phe
                100   105   110

Thr Gly Gln Val Ala Thr Ser Val Ile Gly Ser Asp Ala Phe Gln Glu
                115   120   125

Ala Asp Ile Leu Gly Ile Thr Met Pro Val Thr Lys His Ser Tyr Gln
        130   135   140

Val Arg Gln Pro Glu Asp Leu Pro Arg Ile Ile Lys Glu Ala Phe His
145   150   155   160

Ile Ala Thr Thr Gly Arg Pro Gly Pro Val Leu Ile Asp Ile Pro Lys
                165   170   175

Asp Val Ala Thr Ile Glu Gly Glu Phe Ser Tyr Asp His Glu Met Asn
                180   185   190

Leu Pro Gly Tyr Gln Pro Thr Thr Glu Pro Asn Tyr Leu Gln Ile Arg
                195   200   205

Lys Leu Val Glu Ala Val Ser Ser Ala Lys Lys Pro Val Ile Leu Ala
        210   215   220

Gly Ala Gly Val Leu His Gly Lys Ala Ser Glu Glu Leu Lys Asn Tyr
225   230   235   240

Ala Glu Gln Gln Gln Ile Pro Val Ala His Thr Leu Leu Gly Leu Gly
                245   250   255

Gly Phe Pro Ala Asp His Pro Leu Phe Leu Gly Met Ala Gly Met His
                260   265   270

Gly Thr Tyr Thr Ala Asn Met Ala Leu His Glu Cys Asp Leu Leu Ile
        275   280   285

Ser Ile Gly Ala Arg Phe Asp Asp Arg Val Thr Gly Asn Leu Lys His
        290   295   300

Phe Ala Arg Asn Ala Lys Ile Ala His Ile Asp Ile Asp Pro Ala Glu
305   310   315   320

Ile Gly Lys Ile Met Lys Thr Gln Ile Pro Val Val Gly Asp Ser Lys
                325   330   335

Ile Val Leu Gln Glu Leu Ile Lys Gln Asp Gly Lys Gln Ser Asp Ser
                340   345   350

Ser Glu Trp Lys Lys Gln Leu Ala Glu Trp Lys Glu Glu Tyr Pro Leu
                355   360   365

Trp Tyr Val Asp Asn Glu Glu Glu Gly Phe Lys Pro Gln Lys Leu Ile
        370   375   380

Glu Tyr Ile His Gln Phe Thr Lys Gly Glu Ala Ile Val Ala Thr Asp
385   390   395   400

```
Val Gly Gln His Gln Met Trp Ser Ala Gln Phe Tyr Pro Phe Gln Lys
            405                     410                 415

Ala Asp Lys Trp Val Thr Ser Gly Gly Leu Gly Thr Met Gly Phe Gly
            420                 425                 430

Leu Pro Ala Ala Ile Gly Ala Gln Leu Ala Glu Lys Asp Ala Thr Val
            435                 440                 445

Val Ala Val Val Gly Asp Gly Gly Phe Gln Met Thr Leu Gln Glu Leu
    450                 455                 460

Asp Val Ile Arg Glu Leu Asn Leu Pro Val Lys Val Val Ile Leu Asn
465                 470                 475                 480

Asn Ala Cys Leu Gly Met Val Arg Gln Trp Gln Glu Ile Phe Tyr Glu
            485                 490                 495

Glu Arg Tyr Ser Glu Ser Lys Phe Ala Ser Gln Pro Asp Phe Val Lys
            500                 505                 510

Leu Ser Glu Ala Tyr Gly Ile Lys Gly Ile Arg Ile Ser Ser Glu Ala
            515                 520                 525

Glu Ala Lys Glu Lys Leu Glu Glu Ala Leu Thr Ser Arg Glu Pro Val
    530                 535                 540

Val Ile Asp Val Arg Val Ala Ser Glu Glu Lys Val Phe Pro Met Val
545                 550                 555                 560

Ala Pro Gly Lys Gly Leu His Glu Met Val Gly Val Lys Pro
                565                 570
```

<210> 33
<211> 525
<212> DNA
<213> Bacillus subtilis

<220>
<221> CDS
<222> (1)..(522)

<400> 33
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ttg aaa aga att atc aca ttg act gtg gtg aac cgc tcc ggg gtg tta    48
Met Lys Arg Ile Ile Thr Leu Thr Val Val Asn Arg Ser Gly Val Leu
  1               5                  10                  15

aac cgg atc acc ggt cta ttc aca aaa agg cat tac aac att gaa agc    96
Asn Arg Ile Thr Gly Leu Phe Thr Lys Arg His Tyr Asn Ile Glu Ser
                20                  25                  30

att aca gtt gga cac aca gaa aca gcc ggc gtt tcc aga atc acc ttc   144
Ile Thr Val Gly His Thr Glu Thr Ala Gly Val Ser Arg Ile Thr Phe
            35                  40                  45

gtc gtt cat gtt gaa ggt gaa aat gat gtt gaa cag tta acg aaa cag   192
Val Val His Val Glu Gly Glu Asn Asp Val Glu Gln Leu Thr Lys Gln
    50                  55                  60

ctc aac aaa cag att gat gtg ctg aaa gtc aca gac atc aca aat caa   240
```

```
Leu Asn Lys Gln Ile Asp Val Leu Lys Val Thr Asp Ile Thr Asn Gln
65                  70              75              80


tcg att gtc cag agg gag ctg gcc tta atc aag gtt gtc tcc gca cct    288
Ser Ile Val Gln Arg Glu Leu Ala Leu Ile Lys Val Val Ser Ala Pro
                85                  90                  95


tca aca aga aca gag att aat gga atc ata gaa ccg ttt aga gcc tct    336
Ser Thr Arg Thr Glu Ile Asn Gly Ile Ile Glu Pro Phe Arg Ala Ser
                100                 105                 110


gtc gtt gat gtc agc aga gac agc atc gtt gtt cag gtg aca ggt gaa    384
Val Val Asp Val Ser Arg Asp Ser Ile Val Val Gln Val Thr Gly Glu
            115                 120                 125


tct aac aaa att gaa gcg ctt att gag tta tta aaa cct tat ggc att    432
Ser Asn Lys Ile Glu Ala Leu Ile Glu Leu Leu Lys Pro Tyr Gly Ile
        130                 135                 140


aaa gaa atc gcg aga aca ggt aca acg gct ttt gcg agg gga acc agc    480
Lys Glu Ile Ala Arg Thr Gly Thr Thr Ala Phe Ala Arg Gly Thr Ser
145                 150                 155                 160


aaa agg cgt cat cca ata aaa caa tat cta ttg tat aaa aca taa        525
Lys Arg Arg His Pro Ile Lys Gln Tyr Leu Leu Tyr Lys Thr
                165                 170
```

```
<210> 34
<211> 174
<212> PRT
<213> Bacillus subtilis

<400> 34
Met Lys Arg Ile Ile Thr Leu Thr Val Val Asn Arg Ser Gly Val Leu
 1               5                   10                  15


Asn Arg Ile Thr Gly Leu Phe Thr Lys Arg His Tyr Asn Ile Glu Ser
                20                  25                  30


Ile Thr Val Gly His Thr Glu Thr Ala Gly Val Ser Arg Ile Thr Phe
            35                  40                  45


Val Val His Val Glu Gly Glu Asn Asp Val Glu Gln Leu Thr Lys Gln
        50                  55                  60


Leu Asn Lys Gln Ile Asp Val Leu Lys Val Thr Asp Ile Thr Asn Gln
65                  70                  75                  80


Ser Ile Val Gln Arg Glu Leu Ala Leu Ile Lys Val Val Ser Ala Pro
                85                  90                  95


Ser Thr Arg Thr Glu Ile Asn Gly Ile Ile Glu Pro Phe Arg Ala Ser
                100                 105                 110


Val Val Asp Val Ser Arg Asp Ser Ile Val Val Gln Val Thr Gly Glu
            115                 120                 125


Ser Asn Lys Ile Glu Ala Leu Ile Glu Leu Leu Lys Pro Tyr Gly Ile
        130                 135                 140
```

```
Lys Glu Ile Ala Arg Thr Gly Thr Thr Ala Phe Ala Arg Gly Thr Ser
145                 150                 155                 160

Lys Arg Arg His Pro Ile Lys Gln Tyr Leu Leu Tyr Lys Thr
                165                 170
```

<210> 35
<211> 1029
<212> DNA
<213> Bacillus subtilis

<220>
<221> CDS
<222> (1)..(1026)

<400> 35
```
atg gta aaa gta tat tat aac ggt gat atc aaa gag aac gta ttg gct    48
Met Val Lys Val Tyr Tyr Asn Gly Asp Ile Lys Glu Asn Val Leu Ala
  1               5                  10                  15

gga aaa aca gta gcg gtt atc ggg tac ggt tcg caa ggc cac gca cat    96
Gly Lys Thr Val Ala Val Ile Gly Tyr Gly Ser Gln Gly His Ala His
               20                  25                  30

gcc ctg aac ctt aaa gaa agc gga gta gac gtg atc gtc ggt gtt aga   144
Ala Leu Asn Leu Lys Glu Ser Gly Val Asp Val Ile Val Gly Val Arg
           35                  40                  45

caa gga aaa tct ttc act caa gcc caa gaa gac gga cat aaa gta ttt   192
Gln Gly Lys Ser Phe Thr Gln Ala Gln Glu Asp Gly His Lys Val Phe
       50                  55                  60

tca gta aaa gaa gcg gca gcc caa gcc gaa atc atc atg gtt ctg ctt   240
Ser Val Lys Glu Ala Ala Ala Gln Ala Glu Ile Ile Met Val Leu Leu
 65                  70                  75                  80

ccg gat gag cag cag caa aaa gta tac gaa gct gaa atc aaa gat gaa   288
Pro Asp Glu Gln Gln Gln Lys Val Tyr Glu Ala Glu Ile Lys Asp Glu
                   85                  90                  95

ttg aca gca gga aaa tca tta gta ttc gct cat gga ttt aac gtg cat   336
Leu Thr Ala Gly Lys Ser Leu Val Phe Ala His Gly Phe Asn Val His
               100                 105                 110

ttc cat caa att gtt cct ccg gcg gat gta gat gta ttc tta gtg gcc   384
Phe His Gln Ile Val Pro Pro Ala Asp Val Asp Val Phe Leu Val Ala
           115                 120                 125

cct aaa ggc ccg gga cac ttg gta aga aga aca tat gag caa gga gct   432
Pro Lys Gly Pro Gly His Leu Val Arg Arg Thr Tyr Glu Gln Gly Ala
       130                 135                 140

ggc gta cct gca ttg ttc gca atc tat caa gat gtg act gga gaa gca   480
Gly Val Pro Ala Leu Phe Ala Ile Tyr Gln Asp Val Thr Gly Glu Ala
145                 150                 155                 160

aga gac aaa gcc ctc gct tat gct aaa gga atc ggc ggc gca aga gcg   528
Arg Asp Lys Ala Leu Ala Tyr Ala Lys Gly Ile Gly Gly Ala Arg Ala
                   165                 170                 175
```

```
ggc gta tta gaa acg aca ttt aaa gaa gaa aca gaa aca gat ttg ttc     576
Gly Val Leu Glu Thr Thr Phe Lys Glu Glu Thr Glu Thr Asp Leu Phe
        180             185             190

ggt gag caa gca gtt ctt tgc ggc gga tta agc gcg ctt gtc aaa gcc     624
Gly Glu Gln Ala Val Leu Cys Gly Gly Leu Ser Ala Leu Val Lys Ala
        195             200             205

gga ttt gaa acc tta act gaa gca ggt tat cag cct gaa ctt gca tac     672
Gly Phe Glu Thr Leu Thr Glu Ala Gly Tyr Gln Pro Glu Leu Ala Tyr
    210             215             220

ttc gag tgt ctt cat gag ctg aaa tta atc gta gac ctt atg tac gaa     720
Phe Glu Cys Leu His Glu Leu Lys Leu Ile Val Asp Leu Met Tyr Glu
225             230             235             240

gaa gga ctt gca gga atg aga tat tca atc tct gac aca gca cag tgg     768
Glu Gly Leu Ala Gly Met Arg Tyr Ser Ile Ser Asp Thr Ala Gln Trp
            245             250             255

gga gat ttc gta tca ggc cct cgc gtt gtg gac gcc aaa gta aaa gaa     816
Gly Asp Phe Val Ser Gly Pro Arg Val Val Asp Ala Lys Val Lys Glu
        260             265             270

tct atg aaa gaa gta tta aaa gat atc caa aac ggt aca ttc gca aaa     864
Ser Met Lys Glu Val Leu Lys Asp Ile Gln Asn Gly Thr Phe Ala Lys
        275             280             285

gag tgg atc gtc gaa aac caa gta aac cgt cct cgt ttc aac gct atc     912
Glu Trp Ile Val Glu Asn Gln Val Asn Arg Pro Arg Phe Asn Ala Ile
        290             295             300

aat gca agc gag aac gaa cat caa atc gaa gta gtg gga aga aag ctt     960
Asn Ala Ser Glu Asn Glu His Gln Ile Glu Val Val Gly Arg Lys Leu
305             310             315             320

cgt gaa atg atg ccg ttt gtg aaa caa ggc aag aag aag gaa gcg gtg    1008
Arg Glu Met Met Pro Phe Val Lys Gln Gly Lys Lys Lys Glu Ala Val
            325             330             335

gtc tcc gtt gcg caa aat taa                                        1029
Val Ser Val Ala Gln Asn
            340
```

```
<210> 36
<211> 342
<212> PRT
<213> Bacillus subtilis

<400> 36
Met Val Lys Val Tyr Tyr Asn Gly Asp Ile Lys Glu Asn Val Leu Ala
 1               5              10                  15

Gly Lys Thr Val Ala Val Ile Gly Tyr Gly Ser Gln Gly His Ala His
            20              25                  30

Ala Leu Asn Leu Lys Glu Ser Gly Val Asp Val Ile Val Gly Val Arg
        35              40                  45

Gln Gly Lys Ser Phe Thr Gln Ala Gln Glu Asp Gly His Lys Val Phe
```

```
            50        '                    55                        60
Ser Val Lys Glu Ala Ala Ala Gln Ala Glu Ile Ile Met Val Leu Leu
 65              70                  75                          80

Pro Asp Glu Gln Gln Gln Lys Val Tyr Glu Ala Glu Ile Lys Asp Glu
                85                  90                      95

Leu Thr Ala Gly Lys Ser Leu Val Phe Ala His Gly Phe Asn Val His
            100             105                 110

Phe His Gln Ile Val Pro Pro Ala Asp Val Asp Val Phe Leu Val Ala
        115             120                 125

Pro Lys Gly Pro Gly His Leu Val Arg Arg Thr Tyr Glu Gln Gly Ala
    130             135                 140

Gly Val Pro Ala Leu Phe Ala Ile Tyr Gln Asp Val Thr Gly Glu Ala
145             150                 155                     160

Arg Asp Lys Ala Leu Ala Tyr Ala Lys Gly Ile Gly Gly Ala Arg Ala
            165             170                 175

Gly Val Leu Glu Thr Thr Phe Lys Glu Glu Thr Glu Thr Asp Leu Phe
        180             185                 190

Gly Glu Gln Ala Val Leu Cys Gly Gly Leu Ser Ala Leu Val Lys Ala
        195             200                 205

Gly Phe Glu Thr Leu Thr Glu Ala Gly Tyr Gln Pro Glu Leu Ala Tyr
    210             215                 220

Phe Glu Cys Leu His Glu Leu Lys Leu Ile Val Asp Leu Met Tyr Glu
225             230                 235                     240

Glu Gly Leu Ala Gly Met Arg Tyr Ser Ile Ser Asp Thr Ala Gln Trp
            245             250                 255

Gly Asp Phe Val Ser Gly Pro Arg Val Val Asp Ala Lys Val Lys Glu
        260             265                 270

Ser Met Lys Glu Val Leu Lys Asp Ile Gln Asn Gly Thr Phe Ala Lys
        275             280                 285

Glu Trp Ile Val Glu Asn Gln Val Asn Arg Pro Arg Phe Asn Ala Ile
    290             295                 300

Asn Ala Ser Glu Asn Glu His Gln Ile Glu Val Val Gly Arg Lys Leu
305             310                 315                     320

Arg Glu Met Met Pro Phe Val Lys Gln Gly Lys Lys Lys Glu Ala Val
            325             330                 335

Val Ser Val Ala Gln Asn
            340
```

<210> 37
<211> 1674
<212> DNA
<213> Bacillus subtilis

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<220>
<221> CDS
<222> (1)..(1674)

<400> 37
atg gca gaa tta cgc agt aat atg atc aca caa gga atc gat aga gct      48
Met Ala Glu Leu Arg Ser Asn Met Ile Thr Gln Gly Ile Asp Arg Ala
 1               5                  10                  15

ccg cac cgc agt ttg ctt cgt gca gca ggg gta aaa gaa gag gat ttc      96
Pro His Arg Ser Leu Leu Arg Ala Ala Gly Val Lys Glu Glu Asp Phe
                20                  25                  30

ggc aag ccg ttt att gcg gtg tgt aat tca tac att gat atc gtt ccc     144
Gly Lys Pro Phe Ile Ala Val Cys Asn Ser Tyr Ile Asp Ile Val Pro
            35                  40                  45

ggt cat gtt cac ttg cag gag ttt ggg aaa atc gta aaa gaa gca atc     192
Gly His Val His Leu Gln Glu Phe Gly Lys Ile Val Lys Glu Ala Ile
        50                  55                  60

aga gaa gca ggg ggc gtt ccg ttt gaa ttt aat acc att ggg gta gat     240
Arg Glu Ala Gly Gly Val Pro Phe Glu Phe Asn Thr Ile Gly Val Asp
 65                  70                  75                  80

gat ggc atc gca atg ggg cat atc ggt atg aga tat tcg ctg cca agc     288
Asp Gly Ile Ala Met Gly His Ile Gly Met Arg Tyr Ser Leu Pro Ser
                85                  90                  95

cgt gaa att atc gca gac tct gtg gaa acg gtt gta tcc gca cac tgg     336
Arg Glu Ile Ile Ala Asp Ser Val Glu Thr Val Val Ser Ala His Trp
               100                 105                 110

ttt gac gga atg gtc tgt att ccg aac tgc gac aaa atc aca ccg gga     384
Phe Asp Gly Met Val Cys Ile Pro Asn Cys Asp Lys Ile Thr Pro Gly
           115                 120                 125

atg ctt atg gcg gca atg cgc atc aac att ccg acg att ttt gtc agc     432
Met Leu Met Ala Ala Met Arg Ile Asn Ile Pro Thr Ile Phe Val Ser
       130                 135                 140

ggc gga ccg atg gcg gca gga aga aca agt tac ggg cga aaa atc tcc     480
Gly Gly Pro Met Ala Ala Gly Arg Thr Ser Tyr Gly Arg Lys Ile Ser
145                 150                 155                 160

ctt tcc tca gta ttc gaa ggg gta ggc gcc tac caa gca ggg aaa atc     528
Leu Ser Ser Val Phe Glu Gly Val Gly Ala Tyr Gln Ala Gly Lys Ile
                165                 170                 175

aac gaa aac gag ctt caa gaa cta gag cag ttc gga tgc cca acg tgc     576
Asn Glu Asn Glu Leu Gln Glu Leu Glu Gln Phe Gly Cys Pro Thr Cys
               180                 185                 190

ggg tct tgc tca ggc atg ttt acg gcg aac tca atg aac tgt ctg tca     624
Gly Ser Cys Ser Gly Met Phe Thr Ala Asn Ser Met Asn Cys Leu Ser
           195                 200                 205

gaa gca ctt ggt ctt gct ttg ccg ggt aat gga acc att ctg gca aca     672
Glu Ala Leu Gly Leu Ala Leu Pro Gly Asn Gly Thr Ile Leu Ala Thr
       210                 215                 220
```

```
tct ccg gaa cgc aaa gag ttt gtg aga aaa tcg gct gcg caa tta atg      720
Ser Pro Glu Arg Lys Glu Phe Val Arg Lys Ser Ala Ala Gln Leu Met
225             230             235             240

gaa acg att cgc aaa gat atc aaa ccg cgt gat att gtt aca gta aaa      768
Glu Thr Ile Arg Lys Asp Ile Lys Pro Arg Asp Ile Val Thr Val Lys
            245             250             255

gcg att gat aac gcg ttt gca ctc gat atg gcg ctc gga ggt tct aca      816
Ala Ile Asp Asn Ala Phe Ala Leu Asp Met Ala Leu Gly Gly Ser Thr
            260             265             270

aat acc gtt ctt cat acc ctt gcc ctt gca aac gaa gcc ggc gtt gaa      864
Asn Thr Val Leu His Thr Leu Ala Leu Ala Asn Glu Ala Gly Val Glu
            275             280             285

tac tct tta gaa cgc att aac gaa gtc gct gag cgc gtg ccg cac ttg      912
Tyr Ser Leu Glu Arg Ile Asn Glu Val Ala Glu Arg Val Pro His Leu
    290             295             300

gct aag ctg gcg cct gca tcg gat gtg ttt att gaa gat ctt cac gaa      960
Ala Lys Leu Ala Pro Ala Ser Asp Val Phe Ile Glu Asp Leu His Glu
305             310             315             320

gcg ggc ggc gtt tca gcg gct ctg aat gag ctt tcg aag aaa gaa gga     1008
Ala Gly Gly Val Ser Ala Ala Leu Asn Glu Leu Ser Lys Lys Glu Gly
            325             330             335

gcg ctt cat tta gat gcg ctg act gtt aca gga aaa act ctt gga gaa     1056
Ala Leu His Leu Asp Ala Leu Thr Val Thr Gly Lys Thr Leu Gly Glu
            340             345             350

acc att gcc gga cat gaa gta aag gat tat gac gtc att cac ccg ctg     1104
Thr Ile Ala Gly His Glu Val Lys Asp Tyr Asp Val Ile His Pro Leu
            355             360             365

gat caa cca ttc act gaa aag gga ggc ctt gct gtt tta ttc ggt aat     1152
Asp Gln Pro Phe Thr Glu Lys Gly Gly Leu Ala Val Leu Phe Gly Asn
            370             375             380

cta gct ccg gac ggc gct atc att aaa aca ggc ggc gta cag aat ggg     1200
Leu Ala Pro Asp Gly Ala Ile Ile Lys Thr Gly Gly Val Gln Asn Gly
385             390             395             400

att aca aga cac gaa ggg ccg gct gtc gta ttc gat tct cag gac gag     1248
Ile Thr Arg His Glu Gly Pro Ala Val Val Phe Asp Ser Gln Asp Glu
            405             410             415

gcg ctt gac ggc att atc aac cga aaa gta aaa gaa ggc gac gtt gtc     1296
Ala Leu Asp Gly Ile Ile Asn Arg Lys Val Lys Glu Gly Asp Val Val
            420             425             430

atc atc aga tac gaa ggg cca aaa ggc gga cct ggc atg ccg gaa atg     1344
Ile Ile Arg Tyr Glu Gly Pro Lys Gly Gly Pro Gly Met Pro Glu Met
            435             440             445

ctg gcg cca aca tcc caa atc gtt gga atg gga ctc ggg cca aaa gtg     1392
Leu Ala Pro Thr Ser Gln Ile Val Gly Met Gly Leu Gly Pro Lys Val
450             455             460
```

```
gca ttg att acg gac gga cgt ttt tcc gga gcc tcc cgt ggc ctc tca   1440
Ala Leu Ile Thr Asp Gly Arg Phe Ser Gly Ala Ser Arg Gly Leu Ser
465             470             475             480

atc ggc cac gta tca cct gag gcc gct gag ggc ggg ccg ctt gcc ttt   1488
Ile Gly His Val Ser Pro Glu Ala Ala Glu Gly Gly Pro Leu Ala Phe
                485             490             495

gtt gaa aac gga gac cat att atc gtt gat att gaa aaa cgc atc ttg   1536
Val Glu Asn Gly Asp His Ile Ile Val Asp Ile Glu Lys Arg Ile Leu
                500             505             510

gat gta caa gtg cca gaa gaa gag tgg gaa aaa cga aaa gcg aac tgg   1584
Asp Val Gln Val Pro Glu Glu Glu Trp Glu Lys Arg Lys Ala Asn Trp
            515             520             525

aaa ggt ttt gaa ccg aaa gtg aaa acc ggc tac ctg gca cgt tat tct   1632
Lys Gly Phe Glu Pro Lys Val Lys Thr Gly Tyr Leu Ala Arg Tyr Ser
        530             535             540

aaa ctt gtg aca agt gcc aac acc ggc ggt att atg aaa atc          1674
Lys Leu Val Thr Ser Ala Asn Thr Gly Gly Ile Met Lys Ile
545             550             555
```

```
<210> 38
<211> 558
<212> PRT
<213> Bacillus subtilis

<400> 38
Met Ala Glu Leu Arg Ser Asn Met Ile Thr Gln Gly Ile Asp Arg Ala
 1               5              10              15

Pro His Arg Ser Leu Leu Arg Ala Ala Gly Val Lys Glu Glu Asp Phe
            20              25              30

Gly Lys Pro Phe Ile Ala Val Cys Asn Ser Tyr Ile Asp Ile Val Pro
        35              40              45

Gly His Val His Leu Gln Glu Phe Gly Lys Ile Val Lys Glu Ala Ile
    50              55              60

Arg Glu Ala Gly Gly Val Pro Phe Glu Phe Asn Thr Ile Gly Val Asp
65              70              75              80

Asp Gly Ile Ala Met Gly His Ile Gly Met Arg Tyr Ser Leu Pro Ser
                85              90              95

Arg Glu Ile Ile Ala Asp Ser Val Glu Thr Val Val Ser Ala His Trp
            100             105             110

Phe Asp Gly Met Val Cys Ile Pro Asn Cys Asp Lys Ile Thr Pro Gly
        115             120             125

Met Leu Met Ala Ala Met Arg Ile Asn Ile Pro Thr Ile Phe Val Ser
    130             135             140

Gly Gly Pro Met Ala Ala Gly Arg Thr Ser Tyr Gly Arg Lys Ile Ser
145             150             155             160
```

Leu Ser Ser Val Phe Glu Gly Val Gly Ala Tyr Gln Ala Gly Lys Ile
                165   170   175

Asn Glu Asn Glu Leu Gln Glu Leu Glu Gln Phe Gly Cys Pro Thr Cys
            180   185   190

Gly Ser Cys Ser Gly Met Phe Thr Ala Asn Ser Met Asn Cys Leu Ser
        195   200   205

Glu Ala Leu Gly Leu Ala Leu Pro Gly Asn Gly Thr Ile Leu Ala Thr
        210   215   220

Ser Pro Glu Arg Lys Glu Phe Val Arg Lys Ser Ala Ala Gln Leu Met
225   230   235   240

Glu Thr Ile Arg Lys Asp Ile Lys Pro Arg Asp Ile Val Thr Val Lys
                245   250   255

Ala Ile Asp Asn Ala Phe Ala Leu Asp Met Ala Leu Gly Gly Ser Thr
                260   265   270

Asn Thr Val Leu His Thr Leu Ala Leu Ala Asn Glu Ala Gly Val Glu
            275   280   285

Tyr Ser Leu Glu Arg Ile Asn Glu Val Ala Glu Arg Val Pro His Leu
    290   295   300

Ala Lys Leu Ala Pro Ala Ser Asp Val Phe Ile Glu Asp Leu His Glu
305   310   315   320

Ala Gly Gly Val Ser Ala Ala Leu Asn Glu Leu Ser Lys Lys Glu Gly
                325   330   335

Ala Leu His Leu Asp Ala Leu Thr Val Thr Gly Lys Thr Leu Gly Glu
            340   345   350

Thr Ile Ala Gly His Glu Val Lys Asp Tyr Asp Val Ile His Pro Leu
        355   360   365

Asp Gln Pro Phe Thr Glu Lys Gly Gly Leu Ala Val Leu Phe Gly Asn
    370   375   380

Leu Ala Pro Asp Gly Ala Ile Ile Lys Thr Gly Gly Val Gln Asn Gly
385   390   395   400

Ile Thr Arg His Glu Gly Pro Ala Val Val Phe Asp Ser Gln Asp Glu
                405   410   415

Ala Leu Asp Gly Ile Ile Asn Arg Lys Val Lys Glu Gly Asp Val Val
        420   425   430

Ile Ile Arg Tyr Glu Gly Pro Lys Gly Gly Pro Gly Met Pro Glu Met
        435   440   445

Leu Ala Pro Thr Ser Gln Ile Val Gly Met Gly Leu Gly Pro Lys Val
    450   455   460

Ala Leu Ile Thr Asp Gly Arg Phe Ser Gly Ala Ser Arg Gly Leu Ser
465   470   475   480

Ile Gly His Val Ser Pro Glu Ala Ala Glu Gly Gly Pro Leu Ala Phe

```
                    485                  490                  495
Val Glu Asn Gly Asp His Ile Ile Val Asp Ile Glu Lys Arg Ile Leu
            500                  505                  510

Asp Val Gln Val Pro Glu Glu Glu Trp Glu Lys Arg Lys Ala Asn Trp
            515                  520                  525

Lys Gly Phe Glu Pro Lys Val Lys Thr Gly Tyr Leu Ala Arg Tyr Ser
    530                  535                  540

Lys Leu Val Thr Ser Ala Asn Thr Gly Gly Ile Met Lys Ile
545                  550                  555
```

```
<210> 39
<211> 194
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:promoter
      sequence

<220>
<221> -35_signal
<222> (136)..(141)

<220>
<221> -10_signal
<222> (159)..(164)

<400> 39
gctattgacg acagctatgg ttcactgtcc accaaccaaa actgtgctca gtaccgccaa 60

tatttctccc ttgaggggta caaagaggtg tccctagaag agatccacgc tgtgtaaaaa 120

ttttacaaaa aggtattgac tttccctaca gggtgtgtaa taatttaatt acaggcgggg 180

gcaaccccgc ctgt                                                   194


<210> 40
<211> 163
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:promoter
      sequence

<220>
<221> -35_signal
<222> (113)..(118)

<220>
<221> -10_signal
<222> (136)..(141)

<400> 40
gcctacctag cttccaagaa agatatccta acagcacaag agcggaaaga tgttttgttc 60
```

tacatccaga acaacctctg ctaaaattcc tgaaaaattt tgcaaaaagt tgttgacttt 120

atctacaagg tgtggtataa taatcttaac aacagcagga cgc   163

<210> 41
<211> 127
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:promoter
      sequence

<220>
<221> -35_signal
<222> (34)..(39)

<220>
<221> -10_signal
<222> (58)..(63)

<220>
<221> -35_signal
<222> (75)..(80)

<220>
<221> -10_signal
<222> (98)..(103)

<400> 41
gaggaatcat agaattttgt caaaataatt ttattgacaa cgtcttatta acgttgatat 60

aatttaaatt ttatttgaca aaaatgggct cgtgttgtac aataaatgta gtgaggtgga 120

tgcaatg   127

<210> 42
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:ribosome
      binding site

<400> 42
taaacatgag gaggagaaaa catg   24

<210> 43
<211> 28
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:ribosome
      binding site

<400> 43

attcgagaaa tggagagaat ataatatg   28

<210> 44
<211> 13
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:ribosome
      binding site

<400> 44
agaaaggagg tga   13

<210> 45
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:ribosome
      binding site

<400> 45
ttaagaaagg aggtgannnn atg   23

<210> 46
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:ribosome
      binding site

<400> 46
ttagaaagga ggtgannnnn atg   23

<210> 47
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:ribosome
      binding site

<400> 47
agaaaggagg tgannnnnnn atg   23

<210> 48
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:ribosome
      binding site

```
<400> 48
agaaaggagg tgannnnnna tg                                    22


<210> 49
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:ribosome
     binding site

<400> 49
ccctctagaa ggaggagaaa acatg                                 25


<210> 50
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:ribosome
     binding site

<400> 50
ccctctagag gaggagaaaa catg                                  24


<210> 51
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:ribosome
     binding site

<400> 51
ttagaaagga ggatttaaat atg                                   23


<210> 52
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:ribosome
     binding site

<400> 52
ttagaaagga ggtttaatta atg                                   23


<210> 53
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:ribosome
     binding site

<400> 53
```

ttagaaagga ggtgatttaa atg   23

<210> 54
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:ribosome
      binding site

<400> 54
ttagaaagga ggtgtttaaa atg   23

<210> 55
<211> 28
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:ribosome
      binding site

<400> 55
attcgagaaa ggaggtgaat ataatatg   28

<210> 56
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:ribosome
      binding site

<400> 56
attcgagaaa ggaggtgaat aataatg   27

<210> 57
<211> 28
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:ribosome
      binding site

<400> 57
attcgtagaa aggaggtgaa ttaatatg   28

<210> 58
<211> 3291
<212> DNA
<213> Bacillus subtilis

<400> 58
atggggacta atgtacaggt ggattcagca tctgccgaat gtacacagac gatgagcgga 60

gcattaatgc tgattgaatc attaaaaaaa gagaaagtag aaatgatctt cggttatccg 120

ggcggggctg tgcttccgat ttacgataag ctatacaatt cagggttggt acatatcctt 180

ccccgtcacg aacaaggagc aattcatgca gcggagggat acgcaagggt ctccggaaaa 240

ccgggtgtcg tcattgccac gtcagggccg ggagcgacaa accttgttac aggccttgct 300

gatgccatga ttgattcatt gccgttagtc gtctttacag ggcaggtagc aacctctgta 360

atcgggagcg atgcatttca ggaagcagac attttaggga ttacgatgcc agtaacaaaa 420

cacagctacc aggttcgcca gccggaagat ctgccgcgca tcattaaaga agcgttccat 480

attgcaacaa ctggaagacc cggacctgta ttgattgata ttccgaaaga tgtagcaaca 540

attgaaggag aattcagcta cgatcatgag atgaatctcc cgggatacca gccgacaaca 600

gagccgaatt atttgcagat ccgcaagctt gtggaagccg tgagcagtgc gaaaaaaccg 660

gtgatcctgg cgggtgcggg cgtactgcac ggaaaagcgt cagaagaatt aaaaaattat 720

gctgaacagc agcaaatccc tgtggcacac acccttttgg ggctcggagg cttcccggct 780

gaccatccgc ttttcctagg gatggcggga atgcacggta cttatacagc caatatggcc 840

cttcatgaat gtgatctatt aatcagtatc ggcgcccgtt ttgatgaccg tgtcacagga 900

aacctgaaac actttgccag aaacgcaaag atagcccaca tcgatattga tccagctgaa 960

atcggaaaaa tcatgaaaac acagattcct gtagtcggag acagcaaaat tgtcctgcag 1020

gagctgatca aacaagacgg caaacaaagc gattcaagcg aatggaaaaa acagctcgca 1080

gaatggaaag aagagtatcc gctctggtat gtagataatg aagaagaagg ttttaaacct 1140

cagaaattga ttgaatatat tcatcaattt acaaaaggag aggccattgt cgcaacggat 1200

gtaggccagc atcaaatgtg gtcagcgcaa ttttatccgt tccaaaaagc agataaatgg 1260

gtcacgtcag gcggacttgg aacgatggga ttcggtcttc cggcggcgat cggcgcacag 1320

ctggccgaaa aagatgctac tgttgtcgcg gttgtcggag acggcggatt ccaaatgacg 1380

cttcaagaac tcgatgttat tcgcgaatta aatcttccgg tcaaggtagt gattttaaat 1440

aacgcttgtc tcggaatggt cagacagtgg caggaaattt tctatgaaga acgttattca 1500

gaatctaaat tcgcttctca gcctgacttc gtcaaattgt ccgaagcata cggcattaaa 1560

ggcatcagaa tttcatcaga agcggaagca aaggaaaagc tggaagaggc attaacatca 1620

agagaacctg ttgtcattga cgtgcgggtt gccagcgaag aaaaagtatt cccgatggtg 1680

gctccgggga aagggctgca tgaaatggtg ggggtgaaac cttgaaaaga attatcacat 1740

tgactgtggt gaaccgctcc ggggtgttaa accggatcac cggtctattc acaaaaaggc 1800

attacaacat tgaaagcatt acagttggac acacagaaac agccggcgtt ccagaatca 1860

ccttcgtcgt tcatgttgaa ggtgaaaatg atgttgaaca gttaacgaaa cagctcaaca 1920

```
aacagattga tgtgctgaaa gtcacagaca tcacaaatca atcgattgtc cagagggagc 1980

tggccttaat caaggttgtc tccgcacctt caacaagaac agagattaat ggaatcatag 2040

aaccgtttag agcctctgtc gttgatgtca gcagagacag catcgttgtt caggtgacag 2100

gtgaatctaa caaaattgaa gcgcttattg agttattaaa accttatggc attaaagaaa 2160

tcgcgagaac aggtacaacg gcttttgcga ggggaaccag caaaaggcgt catccaataa 2220

aacaatatct attgtataaa acataacaag ggagagattg aaatggtaaa agtatattat 2280

aacggtgata tcaaagagaa cgtattggct ggaaaaacag tagcggttat cgggtacggt 2340

tcgcaaggcc acgcacatgc cctgaacctt aaagaaagcg gagtagacgt gatcgtcggt 2400

gttagacaag gaaaatcttt cactcaagcc caagaagacg gacataaagt attttcagta 2460

aaagaagcgg cagcccaagc cgaaatcatc atggttctgc ttccggatga gcagcagcaa 2520

aaagtatacg aagctgaaat caaagatgaa ttgacagcag gaaaatcatt agtattcgct 2580

catggattta acgtgcattt ccatcaaatt gttcctccgg cggatgtaga tgtattctta 2640

gtggcccccta aaggcccggg cacttggta agaagaacat atgagcaagg agctggcgta 2700

cctgcattgt tcgcaatcta tcaagatgtg actggagaag caagagacaa agccctcgct 2760

tatgctaaag gaatcggcgg cgcaagagcg ggcgtattag aaacgacatt taaagaagaa 2820

acagaaacag atttgttcgg tgagcaagca gttctttgcg gcggattaag cgcgcttgtc 2880

aaagccggat ttgaaacctt aactgaagca ggttatcagc ctgaacttgc atacttcgag 2940

tgtcttcatg agctgaaatt aatcgtagac cttatgtacg aagaaggact tgcaggaatg 3000

agatattcaa tctctgacac agcacagtgg ggagatttcg tatcaggccc tcgcgttgtg 3060

gacgccaaag taaaagaatc tatgaaagaa gtattaaaag atatccaaaa cggtacattc 3120

gcaaaagagt ggatcgtcga aaaccaagta aaccgtcctc gtttcaacgc tatcaatgca 3180

agcgagaacg aacatcaaat cgaagtagtg ggaagaaagc ttcgtgaaat gatgccgttt 3240

gtgaaacaag gcaagaagaa ggaagcggtg gtctccgttg cgcaaaatta a           3291
```

<210> 59
<211> 2363
<212> DNA
<213> Bacillus subtilis

<220>
<221> CDS
<222> (242)..(1072)

<220>
<221> CDS
<222> (1077)..(1934)

<220>

<221> CDS
<222> (1939)..(2319)

<400> 59
ttggtacaag cccgttgatt ttggtatact tccattgggc agtatcgcct gcgaactgca 60

cctattatta aaatagatag acattgcagc agtctgcctt gatccaaaaa aggactggga 120

cagagggatg aaactcgccg aactttagaa agtgaagaat ccttctcgtt gtaacggaag 180

gttttttggc ttgcagaaga aaacggcaga tcatctcctc taaacatgag gaggagaaaa 240

c atg aaa aca aaa ctg gat ttt cta aaa atg aag gag tct gaa gaa ccg 289
  Met Lys Thr Lys Leu Asp Phe Leu Lys Met Lys Glu Ser Glu Glu Pro
  1   5   10   15

att gtc atg ctg acc gct tat gat tat ccg gca gct aaa ctt gct gaa 337
Ile Val Met Leu Thr Ala Tyr Asp Tyr Pro Ala Ala Lys Leu Ala Glu
                20   25   30

caa gcg gga gtt gac atg att tta gtc ggt gat tca ctt gga atg gtc 385
Gln Ala Gly Val Asp Met Ile Leu Val Gly Asp Ser Leu Gly Met Val
            35   40   45

gtc ctc ggc ctt gat tca act gtc ggt gtg aca gtt gcg gac atg atc 433
Val Leu Gly Leu Asp Ser Thr Val Gly Val Thr Val Ala Asp Met Ile
        50   55   60

cat cat aca aaa gcc gtt aaa agg ggt gcg ccg aat acc ttt att gtg 481
His His Thr Lys Ala Val Lys Arg Gly Ala Pro Asn Thr Phe Ile Val
65   70   75   80

aca gat atg ccg ttt atg tct tat cac ctg tct aag gaa gat acg ctg 529
Thr Asp Met Pro Phe Met Ser Tyr His Leu Ser Lys Glu Asp Thr Leu
                85   90   95

aaa aat gca gcg gct atc gtt cag gaa agc gga gct gac gca ctg aag 577
Lys Asn Ala Ala Ala Ile Val Gln Glu Ser Gly Ala Asp Ala Leu Lys
            100   105   110

ctt gag ggc gga gaa ggc gtg ttt gaa tcc att cgc gca ttg acg ctt 625
Leu Glu Gly Gly Glu Gly Val Phe Glu Ser Ile Arg Ala Leu Thr Leu
            115   120   125

gga ggc att cca gta gtc agt cac tta ggt ttg aca ccg cag tca gtc 673
Gly Gly Ile Pro Val Val Ser His Leu Gly Leu Thr Pro Gln Ser Val
        130   135   140

ggc gta ctg ggc ggc tat aaa gta cag ggc aaa gac gaa caa agc gcc 721
Gly Val Leu Gly Gly Tyr Lys Val Gln Gly Lys Asp Glu Gln Ser Ala
145   150   155   160

aaa aaa tta ata gaa gac agt ata aaa tgc gaa gaa gca gga gct atg 769
Lys Lys Leu Ile Glu Asp Ser Ile Lys Cys Glu Glu Ala Gly Ala Met
                165   170   175

atg ctt gtg ctg gaa tgt gtg ccg gca gaa ctc aca gcc aaa att gcc 817
Met Leu Val Leu Glu Cys Val Pro Ala Glu Leu Thr Ala Lys Ile Ala
                180   185   190

gag acg cta agc ata ccg gtc att gga atc ggg gct ggt gtg aaa gcg 865
Glu Thr Leu Ser Ile Pro Val Ile Gly Ile Gly Ala Gly Val Lys Ala

```
Glu Thr Leu Ser Ile Pro Val Ile Gly Ile Gly Ala Gly Val Lys Ala
    195             200                 205
```

```
gac gga caa gtt ctc gtt tat cat gat att atc ggc cac ggt gtt gag    913
Asp Gly Gln Val Leu Val Tyr His Asp Ile Ile Gly His Gly Val Glu
    210             215                 220
```

```
aga aca cct aaa ttt gta aag caa tat acg cgc att gat gaa acc atc    961
Arg Thr Pro Lys Phe Val Lys Gln Tyr Thr Arg Ile Asp Glu Thr Ile
225             230                 235                 240
```

```
gaa aca gca atc agc gga tat gtt cag gat gta aga cat cgt gct ttc    1009
Glu Thr Ala Ile Ser Gly Tyr Val Gln Asp Val Arg His Arg Ala Phe
                245                 250                 255
```

```
cct gaa caa aag cat tcc ttt caa atg aac cag aca gtg ctt gac ggc    1057
Pro Glu Gln Lys His Ser Phe Gln Met Asn Gln Thr Val Leu Asp Gly
                260                 265                 270
```

```
ttg tac ggg gga aaa taag atg aga cag att act gat att tca cag ctg    1106
Leu Tyr Gly Gly Lys     Met Arg Gln Ile Thr Asp Ile Ser Gln Leu
            275             280                 285
```

```
aaa gaa gcc ata aaa caa tac cat tca gag ggc aag tca atc gga ttt    1154
Lys Glu Ala Ile Lys Gln Tyr His Ser Glu Gly Lys Ser Ile Gly Phe
                290                 295                 300
```

```
gtt ccg acg atg ggg ttt ctg cat gag ggg cat tta acc tta gca gac    1202
Val Pro Thr Met Gly Phe Leu His Glu Gly His Leu Thr Leu Ala Asp
    305                 310                 315
```

```
aaa gca aga caa gaa aac gac gcc gtt att atg agt att ttt gtg aat    1250
Lys Ala Arg Gln Glu Asn Asp Ala Val Ile Met Ser Ile Phe Val Asn
320                 325                 330                 335
```

```
cct gca caa ttc ggc cct aat gaa gat ttt gaa gca tat ccg cgc gat    1298
Pro Ala Gln Phe Gly Pro Asn Glu Asp Phe Glu Ala Tyr Pro Arg Asp
                340                 345                 350
```

```
att gag cgg gat gca gct ctt gca gaa aac gcc gga gtc gat att ctt    1346
Ile Glu Arg Asp Ala Ala Leu Ala Glu Asn Ala Gly Val Asp Ile Leu
                355                 360                 365
```

```
ttt acg cca gat gct cat gat atg tat ccc ggt gaa aag aat gtc acg    1394
Phe Thr Pro Asp Ala His Asp Met Tyr Pro Gly Glu Lys Asn Val Thr
            370                 375                 380
```

```
att cat gta gaa aga cgc aca gac gtg tta tgc ggg cgc tca aga gaa    1442
Ile His Val Glu Arg Arg Thr Asp Val Leu Cys Gly Arg Ser Arg Glu
385                 390                 395
```

```
gga cat ttt gac ggg gtc gcg atc gta ctg acg aag ctt ttc aat cta    1490
Gly His Phe Asp Gly Val Ala Ile Val Leu Thr Lys Leu Phe Asn Leu
400                 405                 410                 415
```

```
gtc aag ccg act cgt gcc tat ttc ggt tta aaa gat gcg cag cag gta    1538
Val Lys Pro Thr Arg Ala Tyr Phe Gly Leu Lys Asp Ala Gln Gln Val
                420                 425                 430
```

```
gct gtt gtt gat ggg tta atc agc gac ttc ttc atg gat att gaa ttg    1586
Ala Val Val Asp Gly Leu Ile Ser Asp Phe Phe Met Asp Ile Glu Leu
```

```
                435                    440                    445

gtt cct gtc gat acg gtc aga gag gaa gac ggc tta gcc aaa agc tct   1634
Val Pro Val Asp Thr Val Arg Glu Glu Asp Gly Leu Ala Lys Ser Ser
            450                 455                 460

cgc aat gta tac tta aca gct gag gaa aga aaa gaa gcg cct aag ctg   1682
Arg Asn Val Tyr Leu Thr Ala Glu Glu Arg Lys Glu Ala Pro Lys Leu
        465                 470                 475

tat cgg gcc ctt caa aca agt gcg gaa ctt gtc caa gcc ggt gaa aga   1730
Tyr Arg Ala Leu Gln Thr Ser Ala Glu Leu Val Gln Ala Gly Glu Arg
480                 485                 490                 495

gat cct gaa gcg gtg ata aaa gct gca aaa gat atc att gaa acg act   1778
Asp Pro Glu Ala Val Ile Lys Ala Ala Lys Asp Ile Ile Glu Thr Thr
                500                 505                 510

agc gga acc ata gac tat gta gag ctt tat tcc tat ccg gaa ctc gag   1826
Ser Gly Thr Ile Asp Tyr Val Glu Leu Tyr Ser Tyr Pro Glu Leu Glu
            515                 520                 525

cct gtg aat gaa att gct gga aag atg att ctc gct gtt gca gtt gct   1874
Pro Val Asn Glu Ile Ala Gly Lys Met Ile Leu Ala Val Ala Val Ala
        530                 535                 540

ttt tca aaa gcg cgt tta ata gat aat atc att att gat att cga gaa   1922
Phe Ser Lys Ala Arg Leu Ile Asp Asn Ile Ile Ile Asp Ile Arg Glu
        545                 550                 555

atg gag aga ata taat atg tat cga aca atg atg agc ggc aaa ctt cac  1971
Met Glu Arg Ile     Met Tyr Arg Thr Met Met Ser Gly Lys Leu His
560                     565                 570

agg gca act gtt acg gaa gca aac ctg aac tat gtg gga agc att aca   2019
Arg Ala Thr Val Thr Glu Ala Asn Leu Asn Tyr Val Gly Ser Ile Thr
575                 580                 585                 590

att gat gaa gat ctc att gat gct gtg gga atg ctt cct aat gaa aaa   2067
Ile Asp Glu Asp Leu Ile Asp Ala Val Gly Met Leu Pro Asn Glu Lys
                595                 600                 605

gta caa att gtg aat aat aat aat gga gca cgt ctt gaa acg tat att   2115
Val Gln Ile Val Asn Asn Asn Asn Gly Ala Arg Leu Glu Thr Tyr Ile
                610                 615                 620

att cct ggt aaa cgg gga agc ggc gtc ata tgc tta aac ggt gca gcc   2163
Ile Pro Gly Lys Arg Gly Ser Gly Val Ile Cys Leu Asn Gly Ala Ala
            625                 630                 635

gca cgc ctt gtg cag gaa gga gat aag gtc att att att tcc tac aaa   2211
Ala Arg Leu Val Gln Glu Gly Asp Lys Val Ile Ile Ile Ser Tyr Lys
        640                 645                 650

atg atg tct gat caa gaa gcg gca agc cat gag ccg aaa gtg gct gtt   2259
Met Met Ser Asp Gln Glu Ala Ala Ser His Glu Pro Lys Val Ala Val
655                 660                 665                 670

ctg aat gat caa aac aaa att gaa caa atg ctg ggg aac gaa cca gcc   2307
Leu Asn Asp Gln Asn Lys Ile Glu Gln Met Leu Gly Asn Glu Pro Ala
                675                 680                 685
```

```
cgt aca att ttg tagaagaaaa gcccccttta tcgggggttt tcttttaaga tttt  2363
Arg Thr Ile Leu
            690
```

<210> 60
<211> 293
<212> PRT
<213> Bacillus subtilis

<400> 60

```
Met Ser Ile Ala Val Ser Glu Glu Glu Ala Lys Ala Val Glu Gly Leu
 1               5                  10                  15

Asn Asp Tyr Leu Ser Val Glu Glu Val Glu Thr Ile Tyr Ile Pro Leu
            20                  25                  30

Val Arg Leu Leu His Leu His Val Lys Ser Ala Ala Glu Arg Asn Lys
        35                  40                  45

His Val Asn Val Phe Leu Lys His Pro His Ser Ala Lys Ile Pro Phe
    50                  55                  60

Ile Ile Gly Ile Ala Gly Ser Val Ala Val Gly Lys Ser Thr Thr Ala
65                  70                  75                  80

Arg Ile Leu Gln Lys Leu Leu Ser Arg Leu Pro Asp Arg Pro Lys Val
                85                  90                  95

Ser Leu Ile Thr Thr Asp Gly Phe Leu Phe Pro Thr Ala Glu Leu Lys
            100                 105                 110

Lys Lys Asn Met Met Ser Arg Lys Gly Phe Pro Glu Ser Tyr Asp Val
        115                 120                 125

Lys Ala Leu Leu Glu Phe Leu Asn Asp Leu Lys Ser Gly Lys Asp Ser
    130                 135                 140

Val Lys Ala Pro Val Tyr Ser His Leu Thr Tyr Asp Arg Glu Glu Gly
145                 150                 155                 160

Val Phe Glu Val Val Glu Gln Ala Asp Ile Val Ile Ile Glu Gly Ile
                165                 170                 175

Asn Val Leu Gln Ser Pro Thr Leu Glu Asp Asp Arg Glu Asn Pro Arg
            180                 185                 190

Ile Phe Val Ser Asp Phe Phe Asp Phe Ser Ile Tyr Val Asp Ala Glu
            195                 200                 205

Glu Ser Arg Ile Phe Thr Trp Tyr Leu Glu Arg Phe Arg Leu Leu Arg
    210                 215                 220

Glu Thr Ala Phe Gln Asn Pro Asp Ser Tyr Phe His Lys Phe Lys Asp
225                 230                 235                 240

Leu Ser Asp Gln Glu Ala Asp Glu Met Ala Ala Ser Ile Trp Glu Ser
                245                 250                 255

Val Asn Arg Pro Asn Leu Tyr Glu Asn Ile Leu Pro Thr Lys Phe Arg
```

```
                  260                    265                      270
      Ser Asp Leu Ile Leu Arg Lys Gly Asp Gly His Lys Val Glu Glu Val
           275                    280                      285

      Leu Val Arg Arg Val
          290
```

<210> 61
<211> 281
<212> PRT
<213> Bacillus subtilis

<400> 61

```
      Met Glu Gly Leu Asn Asp Tyr Leu Ser Val Glu Glu Val Glu Thr Ile
       1                   5                   10                      15

      Tyr Ile Pro Leu Val Arg Leu Leu His Leu His Val Lys Ser Ala Ala
                      20                  25                  30

      Glu Arg Asn Lys His Val Asn Val Phe Leu Lys His Pro His Ser Ala
                  35                  40                  45

      Lys Ile Pro Phe Ile Ile Gly Ile Ala Gly Ser Val Ala Val Gly Lys
          50                  55                  60

      Ser Thr Thr Ala Arg Ile Leu Gln Lys Leu Leu Ser Arg Leu Pro Asp
      65                  70                  75                      80

      Arg Pro Lys Val Ser Leu Ile Thr Thr Asp Gly Phe Leu Phe Pro Thr
                          85                  90                  95

      Ala Glu Leu Lys Lys Lys Asn Met Met Ser Arg Lys Gly Phe Pro Glu
                      100                 105                 110

      Ser Tyr Asp Val Lys Ala Leu Leu Glu Phe Leu Asn Asp Leu Lys Ser
                  115                 120                 125

      Gly Lys Asp Ser Val Lys Ala Pro Val Tyr Ser His Leu Thr Tyr Asp
          130                 135                 140

      Arg Glu Glu Gly Val Phe Glu Val Val Glu Gln Ala Asp Ile Val Ile
      145                 150                 155                 160

      Ile Glu Gly Ile Asn Val Leu Gln Ser Pro Thr Leu Glu Asp Asp Arg
                          165                 170                 175

      Glu Asn Pro Arg Ile Phe Val Ser Asp Phe Phe Asp Phe Ser Ile Tyr
                      180                 185                 190

      Val Asp Ala Glu Glu Ser Arg Ile Phe Thr Trp Tyr Leu Glu Arg Phe
                  195                 200                 205

      Arg Leu Leu Arg Glu Thr Ala Phe Gln Asn Pro Asp Ser Tyr Phe His
          210                 215                 220

      Lys Phe Lys Asp Leu Ser Asp Gln Glu Ala Asp Glu Met Ala Ala Ser
      225                 230                 235                 240

      Ile Trp Glu Ser Val Asn Arg Pro Asn Leu Tyr Glu Asn Ile Leu Pro
```

```
                  245                   250                   255
      Thr Lys Phe Arg Ser Asp Leu Ile Leu Arg Lys Gly Asp Gly His Lys
                  260                   265                   270
      Val Glu Glu Val Leu Val Arg Arg Val
                  275                   280
```

<210> 62
<211> 1092
<212> DNA
<213> Bacillus subtilis

<220>
<221> CDS
<222> (1)..(1089)

<400> 62
```
atg act aaa caa aca att cgc gtt gaa ttg aca tca aca aaa aaa ccg      48
Met Thr Lys Gln Thr Ile Arg Val Glu Leu Thr Ser Thr Lys Lys Pro
 1               5                  10                  15

aaa cca gac cca aat cag ctt tcg ttc gga aga gtg ttt aca gac cac      96
Lys Pro Asp Pro Asn Gln Leu Ser Phe Gly Arg Val Phe Thr Asp His
                20                  25                  30

atg ttt gta atg gac tat gcc gca gat aaa ggt tgg tac gat cca aga     144
Met Phe Val Met Asp Tyr Ala Ala Asp Lys Gly Trp Tyr Asp Pro Arg
            35                  40                  45

atc att cct tat caa ccc tta tca atg gat cca act gca atg gtc tat     192
Ile Ile Pro Tyr Gln Pro Leu Ser Met Asp Pro Thr Ala Met Val Tyr
        50                  55                  60

cac tac ggc caa acc gtg ttt gaa ggg tta aag gct tac gtg tca gag     240
His Tyr Gly Gln Thr Val Phe Glu Gly Leu Lys Ala Tyr Val Ser Glu
 65                  70                  75                  80

gat gac cat gtt ctg ctt ttc aga ccg gaa aaa aat atg gaa cgc ctg     288
Asp Asp His Val Leu Leu Phe Arg Pro Glu Lys Asn Met Glu Arg Leu
                85                  90                  95

aat caa tca aac gac cgc ctc tgc atc ccg caa att gat gaa gaa cag     336
Asn Gln Ser Asn Asp Arg Leu Cys Ile Pro Gln Ile Asp Glu Glu Gln
               100                 105                 110

gtt ctt gaa ggc tta aag cag ctt gtc gca att gat aaa gac tgg att     384
Val Leu Glu Gly Leu Lys Gln Leu Val Ala Ile Asp Lys Asp Trp Ile
           115                 120                 125

cca aat gcg gag ggc acg tcc ctt tac atc cgt ccg ttc atc atc gca     432
Pro Asn Ala Glu Gly Thr Ser Leu Tyr Ile Arg Pro Phe Ile Ile Ala
       130                 135                 140

acc gag cct ttc ctt ggt gtt gcg gca tct cat acg tat aag ctc ttg     480
Thr Glu Pro Phe Leu Gly Val Ala Ala Ser His Thr Tyr Lys Leu Leu
145                 150                 155                 160

atc att ctt tct ccg gtc ggc tct tat tac aaa gaa ggc att aag ccg     528
Ile Ile Leu Ser Pro Val Gly Ser Tyr Tyr Lys Glu Gly Ile Lys Pro
```

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                 165                   170                   175

gtc aaa atc gct gtt gaa agt gaa ttt gtc cgt gcg gta aaa ggc gga    576
Val Lys Ile Ala Val Glu Ser Glu Phe Val Arg Ala Val Lys Gly Gly
            180                   185                   190

aca gga aat gcc aaa acc gca gga aac tat gct tca agc tta aaa gcg    624
Thr Gly Asn Ala Lys Thr Ala Gly Asn Tyr Ala Ser Ser Leu Lys Ala
            195                   200                   205

cag cag gta gcc gaa gag aaa gga ttt tct caa gta ctc tgg ctg gac    672
Gln Gln Val Ala Glu Glu Lys Gly Phe Ser Gln Val Leu Trp Leu Asp
            210                   215                   220

ggc att gag aag aaa tac atc gaa gaa gtc gga agc atg aac atc ttc    720
Gly Ile Glu Lys Lys Tyr Ile Glu Glu Val Gly Ser Met Asn Ile Phe
225                   230                   235                   240

ttc aaa atc aac ggt gaa atc gta aca ccg atg ctg aac ggg agc atc    768
Phe Lys Ile Asn Gly Glu Ile Val Thr Pro Met Leu Asn Gly Ser Ile
                245                   250                   255

ctg gaa ggc att acg cgc aat tca gtc atc gcc ttg ctt aag cat tgg    816
Leu Glu Gly Ile Thr Arg Asn Ser Val Ile Ala Leu Leu Lys His Trp
            260                   265                   270

ggc ctt caa gtt tca gaa cga aaa att gcg atc gat gag gtc atc caa    864
Gly Leu Gln Val Ser Glu Arg Lys Ile Ala Ile Asp Glu Val Ile Gln
            275                   280                   285

gcc cat aaa gac ggc atc ctg gaa gaa gcc ttc gga aca ggt aca gca    912
Ala His Lys Asp Gly Ile Leu Glu Glu Ala Phe Gly Thr Gly Thr Ala
            290                   295                   300

gct gtt att tcc cca gtc ggc gag ctg atc tgg cag gat gaa aca ctt    960
Ala Val Ile Ser Pro Val Gly Glu Leu Ile Trp Gln Asp Glu Thr Leu
305                   310                   315                   320

tcg atc aac aac ggt gaa aca gga gaa atc gca aaa aaa cta tat gac   1008
Ser Ile Asn Asn Gly Glu Thr Gly Glu Ile Ala Lys Lys Leu Tyr Asp
                325                   330                   335

acg att aca ggc att caa aaa ggc gct gtc gca gac gaa ttc gga tgg   1056
Thr Ile Thr Gly Ile Gln Lys Gly Ala Val Ala Asp Glu Phe Gly Trp
            340                   345                   350

acg acc gaa gtc gca gcg ctg act gaa agc aag taa                   1092
Thr Thr Glu Val Ala Ala Leu Thr Glu Ser Lys
            355                   360


<210> 63
<211> 363
<212> PRT
<213> Bacillus subtilis

<400> 63
Met Thr Lys Gln Thr Ile Arg Val Glu Leu Thr Ser Thr Lys Lys Pro
  1               5                  10                  15

Lys Pro Asp Pro Asn Gln Leu Ser Phe Gly Arg Val Phe Thr Asp His
```

```
                        20                          25                          30

Met Phe Val Met Asp Tyr Ala Ala Asp Lys Gly Trp Tyr Asp Pro Arg
            35                  40                  45

Ile Ile Pro Tyr Gln Pro Leu Ser Met Asp Pro Thr Ala Met Val Tyr
        50                  55                  60

His Tyr Gly Gln Thr Val Phe Glu Gly Leu Lys Ala Tyr Val Ser Glu
65              70                  75                  80

Asp Asp His Val Leu Leu Phe Arg Pro Glu Lys Asn Met Glu Arg Leu
                85                  90                  95

Asn Gln Ser Asn Asp Arg Leu Cys Ile Pro Gln Ile Asp Glu Glu Gln
            100                 105                 110

Val Leu Glu Gly Leu Lys Gln Leu Val Ala Ile Asp Lys Asp Trp Ile
            115                 120                 125

Pro Asn Ala Glu Gly Thr Ser Leu Tyr Ile Arg Pro Phe Ile Ile Ala
            130                 135                 140

Thr Glu Pro Phe Leu Gly Val Ala Ala Ser His Thr Tyr Lys Leu Leu
145                 150                 155                 160

Ile Ile Leu Ser Pro Val Gly Ser Tyr Tyr Lys Glu Gly Ile Lys Pro
                165                 170                 175

Val Lys Ile Ala Val Glu Ser Glu Phe Val Arg Ala Val Lys Gly Gly
            180                 185                 190

Thr Gly Asn Ala Lys Thr Ala Gly Asn Tyr Ala Ser Ser Leu Lys Ala
            195                 200                 205

Gln Gln Val Ala Glu Glu Lys Gly Phe Ser Gln Val Leu Trp Leu Asp
        210                 215                 220

Gly Ile Glu Lys Lys Tyr Ile Glu Glu Val Gly Ser Met Asn Ile Phe
225                 230                 235                 240

Phe Lys Ile Asn Gly Glu Ile Val Thr Pro Met Leu Asn Gly Ser Ile
                245                 250                 255

Leu Glu Gly Ile Thr Arg Asn Ser Val Ile Ala Leu Leu Lys His Trp
            260                 265                 270

Gly Leu Gln Val Ser Glu Arg Lys Ile Ala Ile Asp Glu Val Ile Gln
            275                 280                 285

Ala His Lys Asp Gly Ile Leu Glu Glu Ala Phe Gly Thr Gly Thr Ala
        290                 295                 300

Ala Val Ile Ser Pro Val Gly Glu Leu Ile Trp Gln Asp Glu Thr Leu
305                 310                 315                 320

Ser Ile Asn Asn Gly Glu Thr Gly Glu Ile Ala Lys Lys Leu Tyr Asp
                325                 330                 335

Thr Ile Thr Gly Ile Gln Lys Gly Ala Val Ala Asp Glu Phe Gly Trp
            340                 345                 350
```

```
Thr Thr Glu Val Ala Ala Leu Thr Glu Ser Lys
        355                   360
```

<210> 64
<211> 1071
<212> DNA
<213> Bacillus subtilis

<220>
<221> CDS
<222> (1)..(1068)

<400> 64
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ttg aat aag ctt att gaa cga gaa aaa act gta tat tat aag gaa aag    48
Met Asn Lys Leu Ile Glu Arg Glu Lys Thr Val Tyr Tyr Lys Glu Lys
 1               5                  10                  15

ccc gac ccg tct tcc ttg ggg ttt gga caa tat ttt aca gat tat atg    96
Pro Asp Pro Ser Ser Leu Gly Phe Gly Gln Tyr Phe Thr Asp Tyr Met
            20                  25                  30

ttt gtg atg gac tac gaa gag ggg att gga tgg cat cat ccg aga att   144
Phe Val Met Asp Tyr Glu Glu Gly Ile Gly Trp His His Pro Arg Ile
        35                  40                  45

gcg ccg tac gca ccg ctt acg ctt gat ccg tct tca tct gtt ttt cat   192
Ala Pro Tyr Ala Pro Leu Thr Leu Asp Pro Ser Ser Ser Val Phe His
    50                  55                  60

tac ggc cag gct gtt ttt gaa gga tta aaa gca tac aga aca gac gac   240
Tyr Gly Gln Ala Val Phe Glu Gly Leu Lys Ala Tyr Arg Thr Asp Asp
65                  70                  75                  80

ggc agg gtg ctg ctg ttc cgt ccg gat caa aat atc aaa cgg ctg aac   288
Gly Arg Val Leu Leu Phe Arg Pro Asp Gln Asn Ile Lys Arg Leu Asn
                85                  90                  95

aga tcg tgt gag cgc atg agc atg ccc cct tta gac gaa gag ctg gtg   336
Arg Ser Cys Glu Arg Met Ser Met Pro Pro Leu Asp Glu Glu Leu Val
            100                 105                 110

ctt gag gca ttg acg caa tta gtt gag ctg gag aaa gat tgg gtt cca   384
Leu Glu Ala Leu Thr Gln Leu Val Glu Leu Glu Lys Asp Trp Val Pro
        115                 120                 125

aag gaa aaa gga acg tca ctg tat att cgt cct ttt gtc att gcc aca   432
Lys Glu Lys Gly Thr Ser Leu Tyr Ile Arg Pro Phe Val Ile Ala Thr
        130                 135                 140

gaa ccg agt ctc ggt gtg aag gca tcc agg agc tat aca ttt atg atc   480
Glu Pro Ser Leu Gly Val Lys Ala Ser Arg Ser Tyr Thr Phe Met Ile
145                 150                 155                 160

gtg ctt tcg cct gtc ggc tcc tat tat ggc gac gat cag ctg aag ccg   528
Val Leu Ser Pro Val Gly Ser Tyr Tyr Gly Asp Asp Gln Leu Lys Pro
                165                 170                 175

gtt aga atc tat gtc gaa gat gag tat gtg agg gcg gtc aac gga gga   576
Val Arg Ile Tyr Val Glu Asp Glu Tyr Val Arg Ala Val Asn Gly Gly
```

```
                  180                      185                      190
gtc ggg ttt gca aaa acg gct gga aac tat gcc gcc agt ctt cag gca    624
Val Gly Phe Ala Lys Thr Ala Gly Asn Tyr Ala Ala Ser Leu Gln Ala
        195                      200                      205

cag cgg aaa gcg aat gaa ctg ggc tat gac cag gta ctg tgg ctg gac    672
Gln Arg Lys Ala Asn Glu Leu Gly Tyr Asp Gln Val Leu Trp Leu Asp
        210                      215                      220

gcc atc gaa aag aaa tat gtg gaa gaa gta ggg agc atg aac atc ttt    720
Ala Ile Glu Lys Lys Tyr Val Glu Glu Val Gly Ser Met Asn Ile Phe
225                      230                      235                      240

ttc gtc ata aac ggg gaa gct gtc aca cct gct tta agc gga agc att    768
Phe Val Ile Asn Gly Glu Ala Val Thr Pro Ala Leu Ser Gly Ser Ile
                245                      250                      255

tta agc ggg gtt aca cgt gcg tct gcg att gaa ttg att cga agc tgg    816
Leu Ser Gly Val Thr Arg Ala Ser Ala Ile Glu Leu Ile Arg Ser Trp
                260                      265                      270

ggc att ccg gtt cgt gaa gag aga ata tcg att gat gag gtg tat gcg    864
Gly Ile Pro Val Arg Glu Glu Arg Ile Ser Ile Asp Glu Val Tyr Ala
            275                      280                      285

gcc tct gca cgc gga gaa ttg aca gag gtc ttt ggc aca ggc acg gca    912
Ala Ser Ala Arg Gly Glu Leu Thr Glu Val Phe Gly Thr Gly Thr Ala
        290                      295                      300

gca gtc gtt acg cct gtc ggt gaa ctc aac atc cat gga aaa acg gtg    960
Ala Val Val Thr Pro Val Gly Glu Leu Asn Ile His Gly Lys Thr Val
305                      310                      315                      320

att gta ggc gac ggg caa atc ggg gac ctc tcg aaa aag ctg tat gaa   1008
Ile Val Gly Asp Gly Gln Ile Gly Asp Leu Ser Lys Lys Leu Tyr Glu
                325                      330                      335

acg ata aca gat att cag ctt ggc aag gta aaa ggc ccg ttt aac tgg   1056
Thr Ile Thr Asp Ile Gln Leu Gly Lys Val Lys Gly Pro Phe Asn Trp
                340                      345                      350

aca gtg gaa gtg tga                                                1071
Thr Val Glu Val
        355


<210> 65
<211> 356
<212> PRT
<213> Bacillus subtilis

<400> 65
Met Asn Lys Leu Ile Glu Arg Glu Lys Thr Val Tyr Tyr Lys Glu Lys
  1               5                      10                      15

Pro Asp Pro Ser Ser Leu Gly Phe Gly Gln Tyr Phe Thr Asp Tyr Met
            20                      25                      30

Phe Val Met Asp Tyr Glu Glu Gly Ile Gly Trp His His Pro Arg Ile
        35                      40                      45
```

```
Ala Pro Tyr Ala Pro Leu Thr Leu Asp Pro Ser Ser Ser Val Phe His
    50              55          60

Tyr Gly Gln Ala Val Phe Glu Gly Leu Lys Ala Tyr Arg Thr Asp Asp
65              70              75                      80

Gly Arg Val Leu Leu Phe Arg Pro Asp Gln Asn Ile Lys Arg Leu Asn
            85              90                      95

Arg Ser Cys Glu Arg Met Ser Met Pro Pro Leu Asp Glu Glu Leu Val
        100             105         110

Leu Glu Ala Leu Thr Gln Leu Val Glu Leu Glu Lys Asp Trp Val Pro
        115             120         125

Lys Glu Lys Gly Thr Ser Leu Tyr Ile Arg Pro Phe Val Ile Ala Thr
    130             135         140

Glu Pro Ser Leu Gly Val Lys Ala Ser Arg Ser Tyr Thr Phe Met Ile
145             150             155                     160

Val Leu Ser Pro Val Gly Ser Tyr Tyr Gly Asp Asp Gln Leu Lys Pro
            165             170             175

Val Arg Ile Tyr Val Glu Asp Glu Tyr Val Arg Ala Val Asn Gly Gly
            180             185             190

Val Gly Phe Ala Lys Thr Ala Gly Asn Tyr Ala Ala Ser Leu Gln Ala
            195             200             205

Gln Arg Lys Ala Asn Glu Leu Gly Tyr Asp Gln Val Leu Trp Leu Asp
    210             215             220

Ala Ile Glu Lys Lys Tyr Val Glu Glu Val Gly Ser Met Asn Ile Phe
225             230             235                     240

Phe Val Ile Asn Gly Glu Ala Val Thr Pro Ala Leu Ser Gly Ser Ile
            245             250             255

Leu Ser Gly Val Thr Arg Ala Ser Ala Ile Glu Leu Ile Arg Ser Trp
            260             265             270

Gly Ile Pro Val Arg Glu Glu Arg Ile Ser Ile Asp Glu Val Tyr Ala
        275             280             285

Ala Ser Ala Arg Gly Glu Leu Thr Glu Val Phe Gly Thr Gly Thr Ala
    290             295             300

Ala Val Val Thr Pro Val Gly Glu Leu Asn Ile His Gly Lys Thr Val
305             310             315                     320

Ile Val Gly Asp Gly Gln Ile Gly Asp Leu Ser Lys Lys Leu Tyr Glu
            325             330             335

Thr Ile Thr Asp Ile Gln Leu Gly Lys Val Lys Gly Pro Phe Asn Trp
            340             345             350

Thr Val Glu Val
            355
```

```
<210> 66
<211> 1428
<212> DNA
<213> Bacillus subtilis

<220>
<221> CDS
<222> (1)..(1425)

<400> 66
atg tta aac ggc caa aaa gaa tat cgc gtg gaa aaa gac ttc ctt ggg      48
Met Leu Asn Gly Gln Lys Glu Tyr Arg Val Glu Lys Asp Phe Leu Gly
 1               5                  10                  15

gaa aaa caa att gaa gca gat gtt tat tac gga att cag acg ctc cgt      96
Glu Lys Gln Ile Glu Ala Asp Val Tyr Tyr Gly Ile Gln Thr Leu Arg
                20                  25                  30

gct tct gaa aat ttt ccg atc aca gga tac aaa atc cat gag gaa atg     144
Ala Ser Glu Asn Phe Pro Ile Thr Gly Tyr Lys Ile His Glu Glu Met
            35                  40                  45

att aac gca ctg gcg att gtg aaa aaa gct gcg gct ctt gcc aac atg     192
Ile Asn Ala Leu Ala Ile Val Lys Lys Ala Ala Ala Leu Ala Asn Met
        50                  55                  60

gac gtg aaa cgg ctg tat gaa gga att ggc caa gct atc gta caa gcc     240
Asp Val Lys Arg Leu Tyr Glu Gly Ile Gly Gln Ala Ile Val Gln Ala
65                  70                  75                  80

gct gac gag att ctg gaa ggc aag tgg cac gat cag ttt atc gtc gat     288
Ala Asp Glu Ile Leu Glu Gly Lys Trp His Asp Gln Phe Ile Val Asp
                85                  90                  95

ccg att cag ggc ggt gcc gga act tct atg aac atg aac gcg aat gag     336
Pro Ile Gln Gly Gly Ala Gly Thr Ser Met Asn Met Asn Ala Asn Glu
               100                 105                 110

gtt atc gga aac cgg gcg ctt gaa atc atg gga cat aaa aag gga gat     384
Val Ile Gly Asn Arg Ala Leu Glu Ile Met Gly His Lys Lys Gly Asp
           115                 120                 125

tat atc cat tta agt cca aac aca cat gtg aac atg tca cag tct cag     432
Tyr Ile His Leu Ser Pro Asn Thr His Val Asn Met Ser Gln Ser Gln
       130                 135                 140

aac gat gtg ttc ccg act gct atc cat att tcc aca ttg aag ctc tta     480
Asn Asp Val Phe Pro Thr Ala Ile His Ile Ser Thr Leu Lys Leu Leu
145                 150                 155                 160

gaa aaa ctg ctg aaa aca atg gaa gat atg cat agt gtg ttt aaa caa     528
Glu Lys Leu Leu Lys Thr Met Glu Asp Met His Ser Val Phe Lys Gln
               165                 170                 175

aaa gca cag gag ttt cac tct gtt att aaa atg ggc cgg aca cac ctt     576
Lys Ala Gln Glu Phe His Ser Val Ile Lys Met Gly Arg Thr His Leu
           180                 185                 190

caa gat gcg gtt ccg atc cgt ctt ggc cag gaa ttc gaa gct tac agc     624
Gln Asp Ala Val Pro Ile Arg Leu Gly Gln Glu Phe Glu Ala Tyr Ser
```

```
              195                    200                    205

cgt gtt ctc gag cgt gat atc aaa cga atc aag caa tcg cgc cag cac    672
Arg Val Leu Glu Arg Asp Ile Lys Arg Ile Lys Gln Ser Arg Gln His
    210                 215                 220

ctg tat gaa gtc aac atg ggc gca act gct gtt ggt aca ggg ctg aac    720
Leu Tyr Glu Val Asn Met Gly Ala Thr Ala Val Gly Thr Gly Leu Asn
225                 230                 235                 240

gct gat cct gaa tat atc aaa cag gta gta aag cac ctt gct gat att    768
Ala Asp Pro Glu Tyr Ile Lys Gln Val Val Lys His Leu Ala Asp Ile
                245                 250                 255

agc ggg ctt cct ctt gtc ggc gct gat cat ctt gtt gat gcg aca caa    816
Ser Gly Leu Pro Leu Val Gly Ala Asp His Leu Val Asp Ala Thr Gln
                260                 265                 270

aat aca gat gcc tat aca gag gta tca gct tca tta aaa gtc tgc atg    864
Asn Thr Asp Ala Tyr Thr Glu Val Ser Ala Ser Leu Lys Val Cys Met
                275                 280                 285

atg aac atg tcg aag atc gca aac gac ctg cgc tta atg gcg tcg gga    912
Met Asn Met Ser Lys Ile Ala Asn Asp Leu Arg Leu Met Ala Ser Gly
    290                 295                 300

ccg cgc gcc gga ctt gcg gaa att tct ctg cct gca cgt cag ccg ggt    960
Pro Arg Ala Gly Leu Ala Glu Ile Ser Leu Pro Ala Arg Gln Pro Gly
305                 310                 315                 320

tca tct att atg ccg ggg aaa gtc aat ccg gtt atg gcg gag ctg atc   1008
Ser Ser Ile Met Pro Gly Lys Val Asn Pro Val Met Ala Glu Leu Ile
                325                 330                 335

aac caa att gcg ttc cag gtt atc gga aat gac aat aca atc tgc ctt   1056
Asn Gln Ile Ala Phe Gln Val Ile Gly Asn Asp Asn Thr Ile Cys Leu
                340                 345                 350

gct tca gaa gcc ggc cag ctt gag ttg aac gtc atg gag ccc gtg ctt   1104
Ala Ser Glu Ala Gly Gln Leu Glu Leu Asn Val Met Glu Pro Val Leu
            355                 360                 365

gtc ttt aat ttg ctt caa tcc atc agc atc atg aac aac ggc ttc cgt   1152
Val Phe Asn Leu Leu Gln Ser Ile Ser Ile Met Asn Asn Gly Phe Arg
    370                 375                 380

tcg ttc act gac aac tgc tta aaa ggc att gaa gcc aac gaa aag cgt   1200
Ser Phe Thr Asp Asn Cys Leu Lys Gly Ile Glu Ala Asn Glu Lys Arg
385                 390                 395                 400

atg aag caa tac gta gaa aaa agc gca ggc gtg atc aca gct gtc aat   1248
Met Lys Gln Tyr Val Glu Lys Ser Ala Gly Val Ile Thr Ala Val Asn
                405                 410                 415

ccg cat ctt ggg tat gaa gcg gca gct aga att gcc agg gaa gca att   1296
Pro His Leu Gly Tyr Glu Ala Ala Ala Arg Ile Ala Arg Glu Ala Ile
                420                 425                 430

atg aca ggg caa tct gtc cgg gat ctt tgt ctg cag cat gat gtg ctg   1344
Met Thr Gly Gln Ser Val Arg Asp Leu Cys Leu Gln His Asp Val Leu
            435                 440                 445
```

```
act gaa gaa gaa ttg gat att att tta aac cca tat gag atg acc aaa    1392
Thr Glu Glu Glu Leu Asp Ile Ile Leu Asn Pro Tyr Glu Met Thr Lys
    450                 455                 460

cca ggt atc gca ggg aaa gaa cta tta gaa aaa taa                    1428
Pro Gly Ile Ala Gly Lys Glu Leu Leu Glu Lys
465                 470                 475
```

<210> 67
<211> 475
<212> PRT
<213> Bacillus subtilis

<400> 67

```
Met Leu Asn Gly Gln Lys Glu Tyr Arg Val Glu Lys Asp Phe Leu Gly
 1               5                  10                  15

Glu Lys Gln Ile Glu Ala Asp Val Tyr Tyr Gly Ile Gln Thr Leu Arg
            20                  25                  30

Ala Ser Glu Asn Phe Pro Ile Thr Gly Tyr Lys Ile His Glu Glu Met
        35                  40                  45

Ile Asn Ala Leu Ala Ile Val Lys Lys Ala Ala Ala Leu Ala Asn Met
    50                  55                  60

Asp Val Lys Arg Leu Tyr Glu Gly Ile Gly Gln Ala Ile Val Gln Ala
65                  70                  75                  80

Ala Asp Glu Ile Leu Glu Gly Lys Trp His Asp Gln Phe Ile Val Asp
            85                  90                  95

Pro Ile Gln Gly Gly Ala Gly Thr Ser Met Asn Met Asn Ala Asn Glu
            100                 105                 110

Val Ile Gly Asn Arg Ala Leu Glu Ile Met Gly His Lys Lys Gly Asp
        115                 120                 125

Tyr Ile His Leu Ser Pro Asn Thr His Val Asn Met Ser Gln Ser Gln
    130                 135                 140

Asn Asp Val Phe Pro Thr Ala Ile His Ile Ser Thr Leu Lys Leu Leu
145                 150                 155                 160

Glu Lys Leu Leu Lys Thr Met Glu Asp Met His Ser Val Phe Lys Gln
            165                 170                 175

Lys Ala Gln Glu Phe His Ser Val Ile Lys Met Gly Arg Thr His Leu
            180                 185                 190

Gln Asp Ala Val Pro Ile Arg Leu Gly Gln Glu Phe Glu Ala Tyr Ser
            195                 200                 205

Arg Val Leu Glu Arg Asp Ile Lys Arg Ile Lys Gln Ser Arg Gln His
        210                 215                 220

Leu Tyr Glu Val Asn Met Gly Ala Thr Ala Val Gly Thr Gly Leu Asn
225                 230                 235                 240
```

```
Ala Asp Pro Glu Tyr Ile Lys Gln Val Val Lys His Leu Ala Asp Ile
              245             250             255
```

```
Ser Gly Leu Pro Leu Val Gly Ala Asp His Leu Val Asp Ala Thr Gln
              260             265             270
```

```
Asn Thr Asp Ala Tyr Thr Glu Val Ser Ala Ser Leu Lys Val Cys Met
          275             280             285
```

```
Met Asn Met Ser Lys Ile Ala Asn Asp Leu Arg Leu Met Ala Ser Gly
      290             295             300
```

```
Pro Arg Ala Gly Leu Ala Glu Ile Ser Leu Pro Ala Arg Gln Pro Gly
305             310             315             320
```

```
Ser Ser Ile Met Pro Gly Lys Val Asn Pro Val Met Ala Glu Leu Ile
              325             330             335
```

```
Asn Gln Ile Ala Phe Gln Val Ile Gly Asn Asp Asn Thr Ile Cys Leu
              340             345             350
```

```
Ala Ser Glu Ala Gly Gln Leu Glu Leu Asn Val Met Glu Pro Val Leu
          355             360             365
```

```
Val Phe Asn Leu Leu Gln Ser Ile Ser Ile Met Asn Asn Gly Phe Arg
      370             375             380
```

```
Ser Phe Thr Asp Asn Cys Leu Lys Gly Ile Glu Ala Asn Glu Lys Arg
385             390             395             400
```

```
Met Lys Gln Tyr Val Glu Lys Ser Ala Gly Val Ile Thr Ala Val Asn
              405             410             415
```

```
Pro His Leu Gly Tyr Glu Ala Ala Ala Arg Ile Ala Arg Glu Ala Ile
              420             425             430
```

```
Met Thr Gly Gln Ser Val Arg Asp Leu Cys Leu Gln His Asp Val Leu
          435             440             445
```

```
Thr Glu Glu Glu Leu Asp Ile Ile Leu Asn Pro Tyr Glu Met Thr Lys
      450             455             460
```

```
Pro Gly Ile Ala Gly Lys Glu Leu Leu Glu Lys
465             470             475
```

.

```
<210> 68
<211> 768
<212> DNA
<213> Bacillus subtilis

<220>
<221> CDS
<222> (1)..(765)

<400> 68
atg aaa cga gaa agc aac att caa gtg ctc agc cgt ggt caa aaa gat    48
Met Lys Arg Glu Ser Asn Ile Gln Val Leu Ser Arg Gly Gln Lys Asp
 1               5                  10                  15

cag cct gtg agc cag att tat caa gta tca aca atg act tct cta tta   96
```

```
Gln Pro Val Ser Gln Ile Tyr Gln Val Ser Thr Met Thr Ser Leu Leu
            20              25              30

gac gga gta tat gac gga gat ttt gaa ctg tca gag att ccg aaa tat    144
Asp Gly Val Tyr Asp Gly Asp Phe Glu Leu Ser Glu Ile Pro Lys Tyr
            35              40              45

gga gac ttc ggt atc gga acc ttt aac aag ctt gac gga gag ctg att    192
Gly Asp Phe Gly Ile Gly Thr Phe Asn Lys Leu Asp Gly Glu Leu Ile
        50              55              60

ggg ttt gac ggc gaa ttt tac cgt ctt cgc tca gac gga acc gcg aca    240
Gly Phe Asp Gly Glu Phe Tyr Arg Leu Arg Ser Asp Gly Thr Ala Thr
65              70              75              80

ccg gtc caa aat gga gac cgt tca ccg ttc tgt tca ttt acg ttc ttt    288
Pro Val Gln Asn Gly Asp Arg Ser Pro Phe Cys Ser Phe Thr Phe Phe
                85              90              95

aca ccg gac atg acg cac aaa att gat gcg aaa atg aca cgc gaa gac    336
Thr Pro Asp Met Thr His Lys Ile Asp Ala Lys Met Thr Arg Glu Asp
            100             105             110

ttt gaa aaa gag atc aac agc atg ctg cca agc aga aac tta ttt tat    384
Phe Glu Lys Glu Ile Asn Ser Met Leu Pro Ser Arg Asn Leu Phe Tyr
            115             120             125

gca att cgc att gac gga ttg ttt aaa aag gtg cag aca aga aca gta    432
Ala Ile Arg Ile Asp Gly Leu Phe Lys Lys Val Gln Thr Arg Thr Val
            130             135             140

gaa ctt caa gaa aaa cct tac gtg cca atg gtt gaa gcg gtc aaa aca    480
Glu Leu Gln Glu Lys Pro Tyr Val Pro Met Val Glu Ala Val Lys Thr
145             150             155             160

cag ccg att ttc aac ttc gac aac gtg aga gga acg att gta ggt ttc    528
Gln Pro Ile Phe Asn Phe Asp Asn Val Arg Gly Thr Ile Val Gly Phe
                165             170             175

ttg aca cca gct tat gca aac gga atc gcc gtt tct ggc tat cac ctg    576
Leu Thr Pro Ala Tyr Ala Asn Gly Ile Ala Val Ser Gly Tyr His Leu
                180             185             190

cac ttc att gac gaa gga cgc aat tca ggc gga cac gtt ttt gac tat    624
His Phe Ile Asp Glu Gly Arg Asn Ser Gly Gly His Val Phe Asp Tyr
            195             200             205

gtg ctt gag gat tgc acg gtt acg att tct caa aaa atg aac atg aat    672
Val Leu Glu Asp Cys Thr Val Thr Ile Ser Gln Lys Met Asn Met Asn
            210             215             220

ctc aga ctt ccg aac aca gcg gat ttc ttt aat gcg aat ctg gat aac    720
Leu Arg Leu Pro Asn Thr Ala Asp Phe Phe Asn Ala Asn Leu Asp Asn
225             230             235             240

cct gat ttt gcg aaa gat atc gaa aca act gaa gga agc cct gaa taa    768
Pro Asp Phe Ala Lys Asp Ile Glu Thr Thr Glu Gly Ser Pro Glu
            245             250             255
```

<210> 69

<211> 255
<212> PRT
<213> Bacillus subtilis

<400> 69
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Met Lys Arg Glu Ser Asn Ile Gln Val Leu Ser Arg Gly Gln Lys Asp
 1               5                  10                  15

Gln Pro Val Ser Gln Ile Tyr Gln Val Ser Thr Met Thr Ser Leu Leu
                20                  25                  30

Asp Gly Val Tyr Asp Gly Asp Phe Glu Leu Ser Glu Ile Pro Lys Tyr
            35                  40                  45

Gly Asp Phe Gly Ile Gly Thr Phe Asn Lys Leu Asp Gly Glu Leu Ile
        50                  55                  60

Gly Phe Asp Gly Glu Phe Tyr Arg Leu Arg Ser Asp Gly Thr Ala Thr
65                  70                  75                  80

Pro Val Gln Asn Gly Asp Arg Ser Pro Phe Cys Ser Phe Thr Phe Phe
                85                  90                  95

Thr Pro Asp Met Thr His Lys Ile Asp Ala Lys Met Thr Arg Glu Asp
            100                 105                 110

Phe Glu Lys Glu Ile Asn Ser Met Leu Pro Ser Arg Asn Leu Phe Tyr
        115                 120                 125

Ala Ile Arg Ile Asp Gly Leu Phe Lys Lys Val Gln Thr Arg Thr Val
    130                 135                 140

Glu Leu Gln Glu Lys Pro Tyr Val Pro Met Val Glu Ala Val Lys Thr
145                 150                 155                 160

Gln Pro Ile Phe Asn Phe Asp Asn Val Arg Gly Thr Ile Val Gly Phe
                165                 170                 175

Leu Thr Pro Ala Tyr Ala Asn Gly Ile Ala Val Ser Gly Tyr His Leu
            180                 185                 190

His Phe Ile Asp Glu Gly Arg Asn Ser Gly Gly His Val Phe Asp Tyr
        195                 200                 205

Val Leu Glu Asp Cys Thr Val Thr Ile Ser Gln Lys Met Asn Met Asn
    210                 215                 220

Leu Arg Leu Pro Asn Thr Ala Asp Phe Phe Asn Ala Asn Leu Asp Asn
225                 230                 235                 240

Pro Asp Phe Ala Lys Asp Ile Glu Thr Thr Glu Gly Ser Pro Glu
                245                 250                 255
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<210> 70
<211> 1254
<212> DNA
<213> Escherichia coli

<220>
<221> CDS

<222> (1)..(1251)

<400> 70

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atg aca ttc tcc ctt ttt ggt gac aaa ttt acc cgc cac tcc ggc att    48
Met Thr Phe Ser Leu Phe Gly Asp Lys Phe Thr Arg His Ser Gly Ile
  1           5              10                 15

acg ctg ttg atg gaa gat ctg aac gac ggt tta cgc acg cct ggc gcg    96
Thr Leu Leu Met Glu Asp Leu Asn Asp Gly Leu Arg Thr Pro Gly Ala
             20              25                 30

att atg ctc ggc ggc ggt aat ccg gcg cag atc ccg gaa atg cag gac   144
Ile Met Leu Gly Gly Gly Asn Pro Ala Gln Ile Pro Glu Met Gln Asp
            35              40                 45

tac ttc cag acg cta ctg acc gac atg ctg gaa agt ggc aaa gcg act   192
Tyr Phe Gln Thr Leu Leu Thr Asp Met Leu Glu Ser Gly Lys Ala Thr
        50              55                 60

gat gca ctg tgt aac tac gac ggt cca cag ggg aaa acg gag cta ctc   240
Asp Ala Leu Cys Asn Tyr Asp Gly Pro Gln Gly Lys Thr Glu Leu Leu
 65              70                 75                 80

aca ctg ctt gcc gga atg ctg cgc gag aag ttg ggt tgg gat atc gaa   288
Thr Leu Leu Ala Gly Met Leu Arg Glu Lys Leu Gly Trp Asp Ile Glu
                85              90                 95

cca cag aat att gca cta aca aac ggc agc cag agc gcg ttt ttc tac   336
Pro Gln Asn Ile Ala Leu Thr Asn Gly Ser Gln Ser Ala Phe Phe Tyr
            100             105                110

tta ttt aac ctg ttt gcc gga cgc cgt gcc gat ggt cgg gtc aaa aaa   384
Leu Phe Asn Leu Phe Ala Gly Arg Arg Ala Asp Gly Arg Val Lys Lys
            115             120                125

gtg ctg ttc ccg ctt gca ccg gaa tac att ggc tat gct gac gcc gga   432
Val Leu Phe Pro Leu Ala Pro Glu Tyr Ile Gly Tyr Ala Asp Ala Gly
        130             135                140

ctg gaa gaa gat ctg ttt gtc tct gcg cgt ccg aat att gaa ctg ctg   480
Leu Glu Glu Asp Leu Phe Val Ser Ala Arg Pro Asn Ile Glu Leu Leu
145             150             155                160

ccg gaa ggc cag ttt aaa tac cac gtc gat ttt gag cat ctg cat att   528
Pro Glu Gly Gln Phe Lys Tyr His Val Asp Phe Glu His Leu His Ile
            165             170                175

ggc gaa gaa acc ggg atg att tgc gtc tcc cgg ccg acg aat cca aca   576
Gly Glu Glu Thr Gly Met Ile Cys Val Ser Arg Pro Thr Asn Pro Thr
            180             185                190

ggc aat gtg att act gac gaa gag ttg ctg aag ctt gac gcg ctg ggc   624
Gly Asn Val Ile Thr Asp Glu Glu Leu Leu Lys Leu Asp Ala Leu Gly
        195             200                205

aat caa cac ggc att ccg ctg gtg att gat aac gct tat ggc gtc ccg   672
Asn Gln His Gly Ile Pro Leu Val Ile Asp Asn Ala Tyr Gly Val Pro
 210             215                220

ttc ccg ggt atc atc ttc agt gaa gcg cgc ccg cta tgg aat ccg aat   720
Phe Pro Gly Ile Ile Phe Ser Glu Ala Arg Pro Leu Trp Asn Pro Asn
```

```
        225                   230                   235                   240

atc gtg ctg tgc atg agt ctt tcc aag ctg ggt cta cct ggc tcc cgc    768
Ile Val Leu Cys Met Ser Leu Ser Lys Leu Gly Leu Pro Gly Ser Arg
            245               250               255

tgc ggc att atc atc gcc aat gaa aaa atc atc acc gcc atc acc aat    816
Cys Gly Ile Ile Ile Ala Asn Glu Lys Ile Ile Thr Ala Ile Thr Asn
            260               265               270

atg aac ggc att atc agc ctg gca cct ggc ggt att ggt ccg gcg atg    864
Met Asn Gly Ile Ile Ser Leu Ala Pro Gly Gly Ile Gly Pro Ala Met
            275               280               285

atg tgt gaa atg att aag cgt aac gat ctg ctg cgc ctg tct gaa aca    912
Met Cys Glu Met Ile Lys Arg Asn Asp Leu Leu Arg Leu Ser Glu Thr
            290               295               300

gtc atc aaa ccg ttt tac tac cag cgt gtt cag gaa act atc gcc atc    960
Val Ile Lys Pro Phe Tyr Tyr Gln Arg Val Gln Glu Thr Ile Ala Ile
305               310               315               320

att cgc cgc tat tta ccg gaa aat cgc tgc ctg att cat aaa ccg gaa   1008
Ile Arg Arg Tyr Leu Pro Glu Asn Arg Cys Leu Ile His Lys Pro Glu
                325               330               335

gga gcc att ttc ctc tgg cta tgg ttt aag gat ttg ccc att acg acc   1056
Gly Ala Ile Phe Leu Trp Leu Trp Phe Lys Asp Leu Pro Ile Thr Thr
            340               345               350

aag cag ctc tat cag cgc ctg aaa gca cgc ggc gtg ctg atg gtg ccg   1104
Lys Gln Leu Tyr Gln Arg Leu Lys Ala Arg Gly Val Leu Met Val Pro
            355               360               365

ggg cac aac ttc ttc cca ggg ctg gat aaa ccg tgg ccg cat acg cat   1152
Gly His Asn Phe Phe Pro Gly Leu Asp Lys Pro Trp Pro His Thr His
            370               375               380

caa tgt atg cgc atg aac tac gta cca gag ccg gag aaa att gag gcg   1200
Gln Cys Met Arg Met Asn Tyr Val Pro Glu Pro Glu Lys Ile Glu Ala
385               390               395               400

ggg gtg aag att ctg gcg gaa gag ata gaa aga gcc tgg gct gaa agt   1248
Gly Val Lys Ile Leu Ala Glu Glu Ile Glu Arg Ala Trp Ala Glu Ser
            405               410               415

cac taa                                                            1254
His
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<210> 71
<211> 417
<212> PRT
<213> Escherichia coli

<400> 71

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Met Thr Phe Ser Leu Phe Gly Asp Lys Phe Thr Arg His Ser Gly Ile
 1               5                  10                  15

Thr Leu Leu Met Glu Asp Leu Asn Asp Gly Leu Arg Thr Pro Gly Ala
            20                  25                  30
```

Ile Met Leu Gly Gly Gly Asn Pro Ala Gln Ile Pro Glu Met Gln Asp
        35   40   45

Tyr Phe Gln Thr Leu Leu Thr Asp Met Leu Glu Ser Gly Lys Ala Thr
        50   55   60

Asp Ala Leu Cys Asn Tyr Asp Gly Pro Gln Gly Lys Thr Glu Leu Leu
65   70   75   80

Thr Leu Leu Ala Gly Met Leu Arg Glu Lys Leu Gly Trp Asp Ile Glu
                85   90   95

Pro Gln Asn Ile Ala Leu Thr Asn Gly Ser Gln Ser Ala Phe Phe Tyr
                100   105   110

Leu Phe Asn Leu Phe Ala Gly Arg Arg Ala Asp Gly Arg Val Lys Lys
        115   120   125

Val Leu Phe Pro Leu Ala Pro Glu Tyr Ile Gly Tyr Ala Asp Ala Gly
        130   135   140

Leu Glu Glu Asp Leu Phe Val Ser Ala Arg Pro Asn Ile Glu Leu Leu
145   150   155   160

Pro Glu Gly Gln Phe Lys Tyr His Val Asp Phe Glu His Leu His Ile
                165   170   175

Gly Glu Glu Thr Gly Met Ile Cys Val Ser Arg Pro Thr Asn Pro Thr
                180   185   190

Gly Asn Val Ile Thr Asp Glu Glu Leu Leu Lys Leu Asp Ala Leu Gly
        195   200   205

Asn Gln His Gly Ile Pro Leu Val Ile Asp Asn Ala Tyr Gly Val Pro
        210   215   220

Phe Pro Gly Ile Ile Phe Ser Glu Ala Arg Pro Leu Trp Asn Pro Asn
225   230   235   240

Ile Val Leu Cys Met Ser Leu Ser Lys Leu Gly Leu Pro Gly Ser Arg
                245   250   255

Cys Gly Ile Ile Ile Ala Asn Glu Lys Ile Ile Thr Ala Ile Thr Asn
                260   265   270

Met Asn Gly Ile Ile Ser Leu Ala Pro Gly Gly Ile Gly Pro Ala Met
        275   280   285

Met Cys Glu Met Ile Lys Arg Asn Asp Leu Leu Arg Leu Ser Glu Thr
        290   295   300

Val Ile Lys Pro Phe Tyr Tyr Gln Arg Val Gln Glu Thr Ile Ala Ile
305   310   315   320

Ile Arg Arg Tyr Leu Pro Glu Asn Arg Cys Leu Ile His Lys Pro Glu
                325   330   335

Gly Ala Ile Phe Leu Trp Leu Trp Phe Lys Asp Leu Pro Ile Thr Thr
        340   345   350

```
Lys Gln Leu Tyr Gln Arg Leu Lys Ala Arg Gly Val Leu Met Val Pro
        355             360             365

Gly His Asn Phe Phe Pro Gly Leu Asp Lys Pro Trp Pro His Thr His
        370             375             380

Gln Cys Met Arg Met Asn Tyr Val Pro Glu Pro Glu Lys Ile Glu Ala
385                 390             395                 400

Gly Val Lys Ile Leu Ala Glu Glu Ile Glu Arg Ala Trp Ala Glu Ser
                405             410             415

His
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<210> 72
<211> 8803
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Recombinant
      pAN294 plasmid

<400> 72
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tcggtgcggg cctcttcgct attacgccag ctggcgaaag ggggatgtgc tgcaaggcga   120

ttaagttggg taacgccagg gttttcccag tcacgacgtt gtaaaacgac ggccagtgaa   180

ttgtaatacg actcactata gggcgaattg ggcccgacgt cgcatgctgg atgaaaagcc   240

gatgaccgct tttcaggtct gtcagcagct ttttcctgct gtatatgaaa aggaattgtt   300

tttaacgatg tcagaaacgg caggtcacct tgatgtgttg gaggctgaag aagccatcac   360

gtcatattgg gaaggaaata ccgtatactt taaaacaatg aagaggtgaa atgggtgaaa   420

catatagcgg gaaaaaggat ttggataacc ggcgcttcag gagggcttgg agaaagaatc   480

gcatacttat gcgcggctga aggagcccat gtcctgctgt cggctagacg cgaggatcgt   540

ttgatagaaa tcaaaaggaa ataaccgag gaatggagcg acagtgtga gattttttcct   600

ctggatgtcg gccgcctaga ggatatcgcc cgggtccgcg atcagatcgg ctcgattgat   660

gtactgatta acaatgcagg cttcggtata tttgaaacgg ttttagactc tacattggat   720

gacatgaaag cgatgtttga tgtgaatgtc ttcggcctga tcgcctgtac aaaagcggtg   780

cttccgcaaa tgcttgagca aaaaaaggga catatcatca atatcgcctc tcaagcgggg   840

aaaatcgcca caccgaagtc tagcctgtat ccgcgacca aacatgccgt gttaggttac   900

tcaaacgctt tgcggatgga gctttcggga accggcattt atgtgacaac agtcaacccg   960

ggcccgattc agacggactt tttttccatt gctgataaag cgggggacta cgccaaaaat   1020

gtcggccgct ggatgcttga tcctgatgac gtggcagctc aaattacagc tgcaattttt   1080
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```
acgaaaaagc gggagatcaa tcttccgcgt ttaatgaatg ccggcactaa gctgtatcag 1140

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aactgcctgt ggtggagtgg cttgtttctc acggggcagt ttttgatagt ggaagggaga 1260

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tctccgcttt ttgaggtgca gcatatgaag ggaaagctgg aagcggcata tcagcagcca 1560

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gcgaggggcg ggatttatga agtgttaaag tatgctgaaa atctcgcgct tcaagaagga 1680

atgcttcagg aaaccgatga ttaccgcatc ttacaggaag agcggtttac cgggttttc 1740

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ggcgcggcac tcgggtttcg cgtgacagtg catatgtccg ccgatgctaa gcagtggaaa 1860

aaggatctcc tccgccaaaa gggagtcact gttatggagt acgaaacaga ttacagtgaa 1920

gcggtgaacg aagggagacg gcaggcggaa caagatccat tctgttattt tattgatgat 1980

gaacattctc gtcagctgtt cttaggatat gctgttgctg caagccgatt aaaaacacag 2040

cttgactgta tgaatataaa gccaagtctt gagacgccct tgtttgtgta tctgccgtgc 2100

ggagtcggcg gaggaccggg cggtgtagca tttgggctga agcttttata cggagatgat 2160

gttcatgtgt ttttcgcaga accaactcat tcaccttgta tgctgttagg ctttattca 2220

ggacttcacg agaagatctc cgtccaggat atcggcctgg ataatcagac ggctgctgac 2280

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ggtgctgatt tgctaaaaaa acgaaatgga aaataagcag acagtgaaaa ggttttccgt 2640

tacaatcttt gtaagggttt taacctacag agagtcaggt gtaaacagtg aaaaataaag 2700

aacttaacct acatacttta tatacacagc acaatcggga gtcttggtct ggttttgggg 2760

ggcatttgtc gattgctgta tctgaagaag aggcaaaagc tgtggaagga ttgaatgatt 2820

atctatctgt tgaagaagtg gagacgatct atattccgct tgttcgcttg cttcatttac 2880
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atgtcaagtc tgcggctgaa cgcaataagc atgtcaatgt ttttttgaag cacccacatt 2940

cagccaaaat tccgtttatt atcggcattg ccggcagtgt cgcagtcgga aaaagcacga 3000

cggcgcggat cttgcagaag ctgctttcgc gtttgcctga ccgtccaaaa gtgagcctta 3060

tcacgacaga tggtttttta tttcctactg ccgagctgaa aaagaaaaat atgatgtcaa 3120

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Gln Gln Asp Lys Leu Asp Pro Thr Leu Val Glu Gly Val Ile Ile Ser
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Pro Ala Tyr Ala Ile Cys Lys Asn Thr Ile Ser Ser Ile Gln Ser Gly
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Leu Lys Gly Leu Gln Gly Arg Ile Ser Glu Ala Ile Ile Ser Ser Thr
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Gln Gly Val Ile Gly Thr Asn Thr Val Ala Cys Ile Gln Ser Gly Val
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tcccatacaa tcaatagatt gtcgcacctg attgcccgac attatcgcga gcccatttat 3420

acccatataa atcagcatcc atgttggaat ttaatcgcgg cctcgacgag caagacgttt 3480

cccgttgaat atggctcata acacccccttg tattactgtt tatgtaagca gacagtttta 3540

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cggactgaac ggggggttcg tgcatacagt ccagcttgga gcgaactgcc tacccggaac 3960

tgagtgtcag gcgtggaatg agacaaacgc ggccataaca gcggaatgac accggtaaac 4020

cgaaaggcag gaacaggaga gcgcacgagg gagccgccag gggaaacgcc tggtatcttt 4080

atagtcctgt cgggtttcgc caccactgat ttgagcgtca gatttcgtga tgcttgtcag 4140

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<211> 702
<212> DNA
<213> Bacillus subtilis

<220>

<221> CDS
<222> (1)..(699)

<400> 84

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Met	Leu	Leu	Val	Ile	Asp	Val	Gly	Asn	Thr	Asn	Thr	Val	Leu	Gly	Val	
1				5					10					15		

tat	cat	gat	gga	aaa	tta	gaa	tat	cac	tgg	cgt	ata	gaa	aca	agc	agg	96
Tyr	His	Asp	Gly	Lys	Leu	Glu	Tyr	His	Trp	Arg	Ile	Glu	Thr	Ser	Arg	
			20					25					30			

cat	aaa	aca	gaa	gat	gag	ttt	ggg	atg	att	ttg	cgc	tcc	tta	ttt	gat	144
His	Lys	Thr	Glu	Asp	Glu	Phe	Gly	Met	Ile	Leu	Arg	Ser	Leu	Phe	Asp	
		35					40					45				

cac	tcc	ggg	ctt	atg	ttt	gaa	cag	ata	gat	ggc	att	att	att	tcg	tca	192
His	Ser	Gly	Leu	Met	Phe	Glu	Gln	Ile	Asp	Gly	Ile	Ile	Ile	Ser	Ser	
	50					55					60					

gta	gtg	ccg	cca	atc	atg	ttt	gcg	tta	gaa	aga	atg	tgc	aca	aaa	tac	240
Val	Val	Pro	Pro	Ile	Met	Phe	Ala	Leu	Glu	Arg	Met	Cys	Thr	Lys	Tyr	
65					70					75					80	

ttt	cat	atc	gag	cct	caa	att	gtt	ggt	cca	ggt	atg	aaa	acc	ggt	tta	288
Phe	His	Ile	Glu	Pro	Gln	Ile	Val	Gly	Pro	Gly	Met	Lys	Thr	Gly	Leu	
				85					90					95		

aat	ata	aaa	tat	gac	aat	ccg	aaa	gaa	gta	ggg	gca	gac	aga	atc	gta	336
Asn	Ile	Lys	Tyr	Asp	Asn	Pro	Lys	Glu	Val	Gly	Ala	Asp	Arg	Ile	Val	
			100					105					110			

aat	gct	gtc	gct	gcg	ata	cac	ttg	tac	ggc	aat	cca	tta	att	gtt	gtc	384
Asn	Ala	Val	Ala	Ala	Ile	His	Leu	Tyr	Gly	Asn	Pro	Leu	Ile	Val	Val	
		115					120					125				

gat	ttc	gga	acc	gcc	aca	acg	tac	tgc	tat	att	gat	gaa	aac	aaa	caa	432
Asp	Phe	Gly	Thr	Ala	Thr	Thr	Tyr	Cys	Tyr	Ile	Asp	Glu	Asn	Lys	Gln	
	130					135					140					

tac	atg	ggc	ggg	gcg	att	gcc	cct	ggg	att	aca	att	tcg	aca	gag	gcg	480
Tyr	Met	Gly	Gly	Ala	Ile	Ala	Pro	Gly	Ile	Thr	Ile	Ser	Thr	Glu	Ala	
145				150					155					160		

ctt	tac	tcg	cgt	gca	gca	aag	ctt	cct	cgt	atc	gaa	atc	acc	cgg	ccc	528
Leu	Tyr	Ser	Arg	Ala	Ala	Lys	Leu	Pro	Arg	Ile	Glu	Ile	Thr	Arg	Pro	
			165					170					175			

gac	aat	att	atc	gga	aaa	aac	act	gtt	agc	gcg	atg	caa	tct	gga	att	576
Asp	Asn	Ile	Ile	Gly	Lys	Asn	Thr	Val	Ser	Ala	Met	Gln	Ser	Gly	Ile	
			180					185					190			

tta	ttt	ggc	tat	gtc	ggc	caa	gtg	gaa	gga	atc	gtt	aag	cga	atg	aaa	624
Leu	Phe	Gly	Tyr	Val	Gly	Gln	Val	Glu	Gly	Ile	Val	Lys	Arg	Met	Lys	
		195					200					205				

tgg	cag	gca	aaa	cag	gac	cca	agg	tca	ttg	cga	cag	gag	gcc	tgg	cgc	672
Trp	Gln	Ala	Lys	Gln	Asp	Pro	Arg	Ser	Leu	Arg	Gln	Glu	Ala	Trp	Arg	
	210					215					220					

cgc	tca	ttg	cga	acg	aat	cag	att	gta	tag							702

Arg Ser Leu Arg Thr Asn Gln Ile Val
225   230

<210> 85
<211> 233
<212> PRT
<213> Bacillus subtilis

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Tyr His Asp Gly Lys Leu Glu Tyr His Trp Arg Ile Glu Thr Ser Arg
            20   25   30

His Lys Thr Glu Asp Glu Phe Gly Met Ile Leu Arg Ser Leu Phe Asp
        35   40   45

His Ser Gly Leu Met Phe Glu Gln Ile Asp Gly Ile Ile Ile Ser Ser
    50   55   60

Val Val Pro Pro Ile Met Phe Ala Leu Glu Arg Met Cys Thr Lys Tyr
65   70   75   80

Phe His Ile Glu Pro Gln Ile Val Gly Pro Gly Met Lys Thr Gly Leu
            85   90   95

Asn Ile Lys Tyr Asp Asn Pro Lys Glu Val Gly Ala Asp Arg Ile Val
            100   105   110

Asn Ala Val Ala Ala Ile His Leu Tyr Gly Asn Pro Leu Ile Val Val
        115   120   125

Asp Phe Gly Thr Ala Thr Thr Tyr Cys Tyr Ile Asp Glu Asn Lys Gln
    130   135   140

Tyr Met Gly Gly Ala Ile Ala Pro Gly Ile Thr Ile Ser Thr Glu Ala
145   150   155   160

Leu Tyr Ser Arg Ala Ala Lys Leu Pro Arg Ile Glu Ile Thr Arg Pro
            165   170   175

Asp Asn Ile Ile Gly Lys Asn Thr Val Ser Ala Met Gln Ser Gly Ile
            180   185   190

Leu Phe Gly Tyr Val Gly Gln Val Glu Gly Ile Val Lys Arg Met Lys
            195   200   205

Trp Gln Ala Lys Gln Asp Pro Arg Ser Leu Arg Gln Glu Ala Trp Arg
    210   215   220

Arg Ser Leu Arg Thr Asn Gln Ile Val
225   230

<210> 86
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<212> DNA
<213> Bacillus subtilis

<220>

<221> CDS
<222> (1)..(1620)

<400> 86

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 1               5                  10                  15

tac aag ata aag gac ctg aaa tta tcg ttg ccc ggc acg aac aaa acg    96
Tyr Lys Ile Lys Asp Leu Lys Leu Ser Leu Pro Gly Thr Asn Lys Thr
            20                  25                  30

cag caa ttc atg gcc caa gca gtc ggc cgt tta act gga aaa ccg gga   144
Gln Gln Phe Met Ala Gln Ala Val Gly Arg Leu Thr Gly Lys Pro Gly
        35                  40                  45

gtc gtg tta gtc aca tca gga ccg ggt gcc tct aac ttg gca aca ggc   192
Val Val Leu Val Thr Ser Gly Pro Gly Ala Ser Asn Leu Ala Thr Gly
    50                  55                  60

ctg ctg aca gcg aac act gaa gga gac cct gtc gtt gcg ctt gct gga   240
Leu Leu Thr Ala Asn Thr Glu Gly Asp Pro Val Val Ala Leu Ala Gly
65                  70                  75                  80

aac gtg atc cgt gca tat cgt tta aaa cgg aca cat caa tct ttg gat   288
Asn Val Ile Arg Ala Tyr Arg Leu Lys Arg Thr His Gln Ser Leu Asp
                85                  90                  95

aat gcg gcg cta ttc cag ccg att aca aaa tac agt gta gaa gtt caa   336
Asn Ala Ala Leu Phe Gln Pro Ile Thr Lys Tyr Ser Val Glu Val Gln
            100                 105                 110

gat gta aaa aat ata ccg gaa gct gtt aca aat gca ttt agg ata gcg   384
Asp Val Lys Asn Ile Pro Glu Ala Val Thr Asn Ala Phe Arg Ile Ala
        115                 120                 125

tca gca ggg cag gct ggg gcc gct ttt gtg agc ttt ccg caa gat gtt   432
Ser Ala Gly Gln Ala Gly Ala Ala Phe Val Ser Phe Pro Gln Asp Val
    130                 135                 140

gtg aat gaa gtc aca aat acg aaa aac gtg cgt gct gtt gca gcg cca   480
Val Asn Glu Val Thr Asn Thr Lys Asn Val Arg Ala Val Ala Ala Pro
145                 150                 155                 160

aaa ctc ggt cct gca gca gat gat gca atc agt gcg gcc ata gca aaa   528
Lys Leu Gly Pro Ala Ala Asp Asp Ala Ile Ser Ala Ala Ile Ala Lys
                165                 170                 175

atc caa aca gca aaa ctt cct gtc gtt ttg gtc ggc atg aaa ggc gga   576
Ile Gln Thr Ala Lys Leu Pro Val Val Leu Val Gly Met Lys Gly Gly
            180                 185                 190

aga ccg gaa gca att aaa gcg gtt cgc aag ctt ttg aaa aag gtt cag   624
Arg Pro Glu Ala Ile Lys Ala Val Arg Lys Leu Leu Lys Lys Val Gln
        195                 200                 205

ctt cca ttt gtt gaa aca tat caa gct gcc ggt acc ctt tct aga gat   672
Leu Pro Phe Val Glu Thr Tyr Gln Ala Ala Gly Thr Leu Ser Arg Asp
    210                 215                 220

tta gag gat caa tat ttt ggc cgt atc ggt ttg ttc cgc aac cag cct   720
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Leu Glu Asp Gln Tyr Phe Gly Arg Ile Gly Leu Phe Arg Asn Gln Pro
225             230             235             240


ggc gat tta ctg cta gag cag gca gat gtt gtt ctg acg atc ggc tat     768
Gly Asp Leu Leu Leu Glu Gln Ala Asp Val Val Leu Thr Ile Gly Tyr
                245             250             255


gac ccg att gaa tat gat ccg aaa ttc tgg aat atc aat gga gac cgg     816
Asp Pro Ile Glu Tyr Asp Pro Lys Phe Trp Asn Ile Asn Gly Asp Arg
            260             265             270


aca att atc cat tta gac gag att atc gct gac att gat cat gct tac     864
Thr Ile Ile His Leu Asp Glu Ile Ile Ala Asp Ile Asp His Ala Tyr
            275             280             285


cag cct gat ctt gaa ttg atc ggt gac att ccg tcc acg atc aat cat     912
Gln Pro Asp Leu Glu Leu Ile Gly Asp Ile Pro Ser Thr Ile Asn His
    290             295             300


atc gaa cac gat gct gtg aaa gtg gaa ttt gca gag cgt gag cag aaa     960
Ile Glu His Asp Ala Val Lys Val Glu Phe Ala Glu Arg Glu Gln Lys
305             310             315             320


atc ctt tct gat tta aaa caa tat atg cat gaa ggt gag cag gtg cct    1008
Ile Leu Ser Asp Leu Lys Gln Tyr Met His Glu Gly Glu Gln Val Pro
                325             330             335


gca gat tgg aaa tca gac aga gcg cac cct ctt gaa atc gtt aaa gag    1056
Ala Asp Trp Lys Ser Asp Arg Ala His Pro Leu Glu Ile Val Lys Glu
            340             345             350


ttg cgt aat gca gtc gat gat cat gtt aca gta act tgc gat atc ggt    1104
Leu Arg Asn Ala Val Asp Asp His Val Thr Val Thr Cys Asp Ile Gly
            355             360             365


tcg cac tcc att tgg atg tca cgt tat ttc cgc agc tac gag ccg tta    1152
Ser His Ser Ile Trp Met Ser Arg Tyr Phe Arg Ser Tyr Glu Pro Leu
    370             375             380


aca tta atg atc agt aac ggt atg caa aca ctc ggc gtt gcg ctt cct    1200
Thr Leu Met Ile Ser Asn Gly Met Gln Thr Leu Gly Val Ala Leu Pro
385             390             395             400


tgg gca atc ggc gct tca ttg gtg aaa ccg gga gaa aaa gtg gtt tct    1248
Trp Ala Ile Gly Ala Ser Leu Val Lys Pro Gly Glu Lys Val Val Ser
                405             410             415


gtc tct ggt gac ggc ggt ttc tta ttc tca gca atg gaa tta gag aca    1296
Val Ser Gly Asp Gly Gly Phe Leu Phe Ser Ala Met Glu Leu Glu Thr
                420             425             430


gca gtt cga cta aaa gca cca att gta cac att gta tgg aac gac agc    1344
Ala Val Arg Leu Lys Ala Pro Ile Val His Ile Val Trp Asn Asp Ser
            435             440             445


aca tat gac atg gtg cat ttc cag caa ttg aaa aaa tat aac cgt aca    1392
Thr Tyr Asp Met Val His Phe Gln Gln Leu Lys Lys Tyr Asn Arg Thr
    450             455             460


tct gcg gtc gat ttc gga aat atc gat atc gtg aaa tat gcg gaa agc    1440
Ser Ala Val Asp Phe Gly Asn Ile Asp Ile Val Lys Tyr Ala Glu Ser
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465                    470                    475                    480

ttc gga gca act gcg ttg cgc gta gaa tca cca gac cag ctg gca gat    1488
Phe Gly Ala Thr Ala Leu Arg Val Glu Ser Pro Asp Gln Leu Ala Asp
            485                490                495

gtt ctg cgt caa ggc atg aac gct gaa ggt cct gtc atc atc gat gtc    1536
Val Leu Arg Gln Gly Met Asn Ala Glu Gly Pro Val Ile Ile Asp Val
            500                505                510

ccg gtt gac tac agt gat aac att aat tta gca agt gac aag ctt ccg    1584
Pro Val Asp Tyr Ser Asp Asn Ile Asn Leu Ala Ser Asp Lys Leu Pro
            515                520                525

aaa gaa ttc ggg gaa ctc atg aaa acg aaa gct ctc tag            1623
Lys Glu Phe Gly Glu Leu Met Lys Thr Lys Ala Leu
    530                535                540
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<210> 87
<211> 540
<212> PRT
<213> Bacillus subtilis

<400> 87

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Met Tyr Leu Ala Phe Gln Val Gln Lys Leu Met Arg Tyr Leu Thr Leu
 1               5                10                15

Tyr Lys Ile Lys Asp Leu Lys Leu Ser Leu Pro Gly Thr Asn Lys Thr
            20                25                30

Gln Gln Phe Met Ala Gln Ala Val Gly Arg Leu Thr Gly Lys Pro Gly
        35                40                45

Val Val Leu Val Thr Ser Gly Pro Gly Ala Ser Asn Leu Ala Thr Gly
    50                55                60

Leu Leu Thr Ala Asn Thr Glu Gly Asp Pro Val Val Ala Leu Ala Gly
65                70                75                80

Asn Val Ile Arg Ala Tyr Arg Leu Lys Arg Thr His Gln Ser Leu Asp
            85                90                95

Asn Ala Ala Leu Phe Gln Pro Ile Thr Lys Tyr Ser Val Glu Val Gln
            100                105                110

Asp Val Lys Asn Ile Pro Glu Ala Val Thr Asn Ala Phe Arg Ile Ala
            115                120                125

Ser Ala Gly Gln Ala Gly Ala Ala Phe Val Ser Phe Pro Gln Asp Val
    130                135                140

Val Asn Glu Val Thr Asn Thr Lys Asn Val Arg Ala Val Ala Ala Pro
145                150                155                160

Lys Leu Gly Pro Ala Ala Asp Asp Ala Ile Ser Ala Ala Ile Ala Lys
            165                170                175

Ile Gln Thr Ala Lys Leu Pro Val Val Leu Val Gly Met Lys Gly Gly
            180                185                190
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Arg Pro Glu Ala Ile Lys Ala Val Arg Lys Leu Leu Lys Lys Val Gln
        195   200   205

Leu Pro Phe Val Glu Thr Tyr Gln Ala Ala Gly Thr Leu Ser Arg Asp
        210   215   220

Leu Glu Asp Gln Tyr Phe Gly Arg Ile Gly Leu Phe Arg Asn Gln Pro
225   230   235   240

Gly Asp Leu Leu Leu Glu Gln Ala Asp Val Val Leu Thr Ile Gly Tyr
                245   250   255

Asp Pro Ile Glu Tyr Asp Pro Lys Phe Trp Asn Ile Asn Gly Asp Arg
        260   265   270

Thr Ile Ile His Leu Asp Glu Ile Ile Ala Asp Ile Asp His Ala Tyr
        275   280   285

Gln Pro Asp Leu Glu Leu Ile Gly Asp Ile Pro Ser Thr Ile Asn His
        290   295   300

Ile Glu His Asp Ala Val Lys Val Glu Phe Ala Glu Arg Glu Gln Lys
305   310   315   320

Ile Leu Ser Asp Leu Lys Gln Tyr Met His Glu Gly Glu Gln Val Pro
                325   330   335

Ala Asp Trp Lys Ser Asp Arg Ala His Pro Leu Glu Ile Val Lys Glu
                340   345   350

Leu Arg Asn Ala Val Asp Asp His Val Thr Val Thr Cys Asp Ile Gly
                355   360   365

Ser His Ser Ile Trp Met Ser Arg Tyr Phe Arg Ser Tyr Glu Pro Leu
        370   375   380

Thr Leu Met Ile Ser Asn Gly Met Gln Thr Leu Gly Val Ala Leu Pro
385   390   395   400

Trp Ala Ile Gly Ala Ser Leu Val Lys Pro Gly Glu Lys Val Val Ser
                405   410   415

Val Ser Gly Asp Gly Gly Phe Leu Phe Ser Ala Met Glu Leu Glu Thr
                420   425   430

Ala Val Arg Leu Lys Ala Pro Ile Val His Ile Val Trp Asn Asp Ser
                435   440   445

Thr Tyr Asp Met Val His Phe Gln Gln Leu Lys Lys Tyr Asn Arg Thr
        450   455   460

Ser Ala Val Asp Phe Gly Asn Ile Asp Ile Val Lys Tyr Ala Glu Ser
465   470   475   480

Phe Gly Ala Thr Ala Leu Arg Val Glu Ser Pro Asp Gln Leu Ala Asp
                485   490   495

Val Leu Arg Gln Gly Met Asn Ala Glu Gly Pro Val Ile Ile Asp Val
                500   505   510

Pro Val Asp Tyr Ser Asp Asn Ile Asn Leu Ala Ser Asp Lys Leu Pro

                515   520   525

Lys Glu Phe Gly Glu Leu Met Lys Thr Lys Ala Leu
        530   535   540

<210> 88
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: ribosome
        binding site

<220>
<223> All occurrences of n indicate any nucleotide

<400> 88
agaaaggagg tgannnnnnn atg   23

<210> 89
<211> 7
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<213> Artificial Sequence

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<223> Description of Artificial Sequence: PanC
        C terminus

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<210> 90
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<213> Artificial Sequence

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<223> Description of Artificial Sequence: PanC
        C terminus

<400> 90
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<210> 91
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<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: PanC
        C terminus

<400> 91
Ile Arg Arg Lys Glu Val Asn

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        1                      5

<210> 92
<211> 6688
<212> DNA
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<220>
<223> Description of Artificial Sequence: Recombinant
      pAN336 plasmid

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ttaagttggg taacgccagg gttttcccag tcacgacgtt gtaaaacgac ggccagtgaa 180

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aaaaaaatcg atgcgcgtga tattgacgaa gcgacggacc gtgtaattgc cggacccgct 360

aagaagagcc gcgttatctc caagaaagaa cgcaatatcg tggcttatca cgaaggcgga 420

cacaccgtta tcggtctcgt tttagatgag gcagatatgg ttcataaagt aacgattgtt 480

cctcggggcc aggctggcgg ttatgctgtt atgctgccaa gagaagaccg ttatttccaa 540

acaaagccgg agctgcttga taaaattgtc ggcctcttgg gcggacgtgt tgctgaagag 600

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gcaagacgaa tggttacaga attcggtatg tcagaaaaac tgggaccgtt gcaatttgga 720

cagtctcagg gcggtcaggt attcttaggc cgtgatttca acaacgaaca gaactacagt 780

gatcaaatcg cttacgaaat tgatcaggaa attcagcgca tcatcaaaga atgttatgag 840

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ccggctgacg ctggcagttt ttttatgtaa atgattggct cagctgcggc ttttacaatc 1140

atccaattct ggtatcgatt tgtttacaaa tgagccgctg atcgtgtatg gtattgtaga 1200

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ttatcgatgt ggggaacacc ctgcagctcg agtgaaatac cgcacagatg cgtaaggaga 1320

aaataccgca tcaggcgata aacccagcga accatttgag gtgataggta agattatacc 1380

gaggtatgaa aacgagaatt ggacctttac agaattactc tatgaagcgc catatttaaa 1440

aagctaccaa gacgaagagg atgaagagga tgaggaggca gattgccttg aatatattga 1500
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acttgcatgg actaatgctt gaaacccagg acaataacct tatagcttgt aaattctatc 1680

ataattgtgg tttcaaaatc ggctccgtcg atactatgtt atacgccaac tttcaaaaca 1740

actttgaaaa agctgttttc tggtatttaa ggttttagaa tgcaaggaac agtgaattgg 1800

agttcgtctt gttataatta gcttcttggg gtatctttaa atactgtaga aaagaggaag 1860

gaaataataa atggctaaaa tgagaatatc accggaattg aaaaaactga tcgaaaaata 1920

ccgctgcgta aaagatacgg aaggaatgtc tcctgctaag gtatataagc tggtgggaga 1980

aaatgaaaac ctatatttaa aaatgacgga cagccggtat aaagggacca cctatgatgt 2040

ggaacgggaa aaggacatga tgctatggct ggaaggaaag ctgcctgttc caaaggtcct 2100

gcactttgaa cggcatgatg gctggagcaa tctgctcatg agtgaggccg atggcgtcct 2160

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tattttactg gatgaattgt tttagtacct agatttagat gtctaaaaag ctttaactac 2700

aagctttta gacatctaat ctttctgaa gtacatccgc aactgtccat actctgatgt 2760

tttatatctt ttctaaaagt tcgctagata ggggtcccga gcgcctacga ggaatttgta 2820

tcgccattcg ccattcaggc tgcgcaactg ttgggaaggg cgatcggtgc ggtcgactgg 2880

caggcaaaac aggacccaag gtcattgcga caggaggcct ggcgccgctc attgcgaacg 2940

aatcagattg tatagacatc gttgatccat tcttaaccct aaaagggctg gaattgattt 3000

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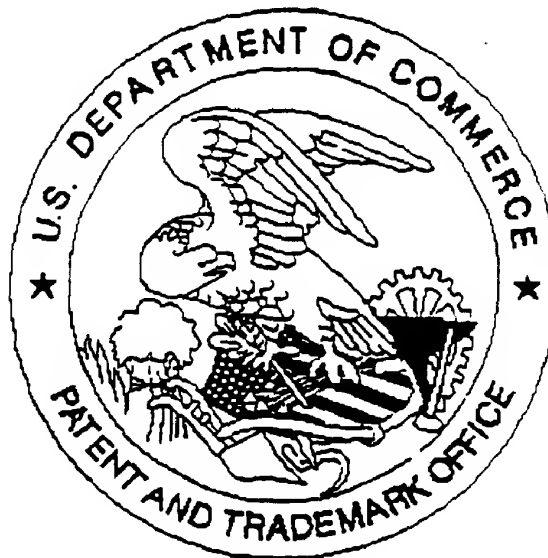
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